Supporting Information

***Rec. Nat. Prod.* 10:5 (2016) 649-653**

Cytotoxic and Antibacterial Activities of Constituents from *Calophyllum ferrugineum* Ridley

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Experimental Details

Cytotoxic Activity:

The cytotoxic activity was evaluated by MTT colorimetric assay [1,2]. The sample stock solution (100 µg/mL) was dissolve in 1% (v/v) DMSO in phosphate buffered saline (PBS). The samples were further diluted with DMEM to afford concentration ranging from 100 – 3.13 µg/mL obtained from twofold dilution. The cells were cultured in Dulbecco's modified Eagle's Medium (DMEM) media supplemented with 10% fetal bovine serum and 2% penicillin-streptomycin. In brief, 90 µL of cell suspension in DMEM were seeded in 96-well microplate and was counted directly by using trypan blue dye. The cells were treated with samples after reaching confluence (2 x 10^5 cell/mL) and were pre-incubated at 37ºC in humidified atmosphere with 5% CO_2 for 24 hours. 20 µL of MTT (5 mg/mL in PBS) was added to all well in dark condition and pre-incubated for another 4 hours. 100 µL of DMSO was added to all well to solubilize the water-insoluble purple formazan crystal formed and pre-incubated in dark condition at room temperature. The absorbance was read after 1 hour at 570 nm and 630 nm as the reference wavelength. Untreated cells served as control group and considered as 100% of viable cells. Results were expressed as percentage of cell viability of samples relative to the untreated control cell following the formula;

$$\%\text{Inhibition Concentration (\%IC)} = [(A_{sample} - A_{MTT\ blank})/ (A_{control} - A_{MTT\ blank})] \times 100\%\%$$

where A_{sample} is the absorbance of cells treated with samples, $A_{MTT\ blank}$ is the absorbance of MTT reagent with DMSO only and $A_{control}$ is the absorbance of untreated control cells.

Antibacterial Activity:

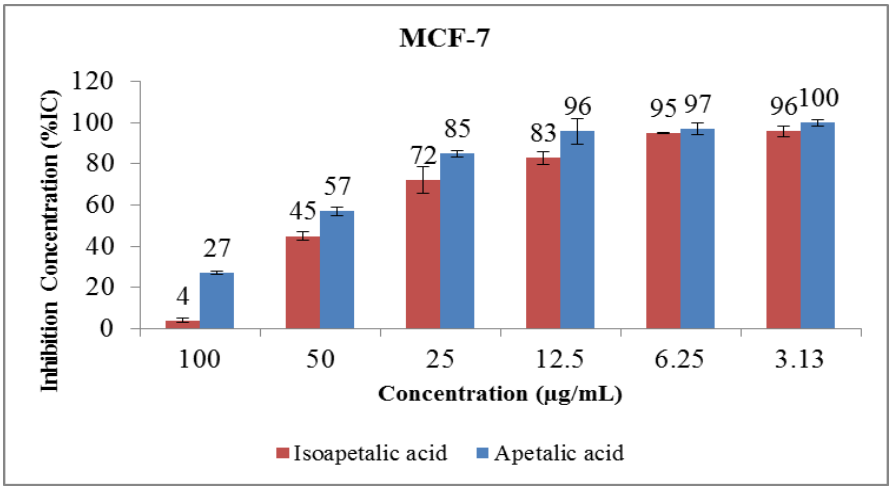
The antibacterial activity of all compounds was tested quantitatively by evaluating their minimum inhibition concentration (MIC). The MIC was carried out by micro-broth dilution [3–7]. The sample stock solution (1000 µg/mL) was prepared in 5% DMSO in nutrient broth (NB) supplemented with 0.02% (v/v) Tween 80. Further twofold dilution with NB was performed to afford concentration of samples from 100 – 7.81 µg/mL. 50 µL of bacteria inocula (10^6 CFU/mL) was dispensed in the 96-well microplate followed by 50 µL of the sample solution. The microplates were pre-incubated for 24 hour at 37ºC for *S. aureus, E. coli* and *P. aeruginosa* and 30 ºC *for B. subtilis*. 25 µL of 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium (INT) (0.2 mg/mL in distilled water) solution was added to all wells and were further pre-incubated for at least 30 minutes. Bacteria growth in the wells was indicated by formation of reddish-pink colour while clear well indicates inhibition of bacteria growth by the sample. Streptomycin sulphate was employed as positive control in this assay.

Statistical Analysis of Data:

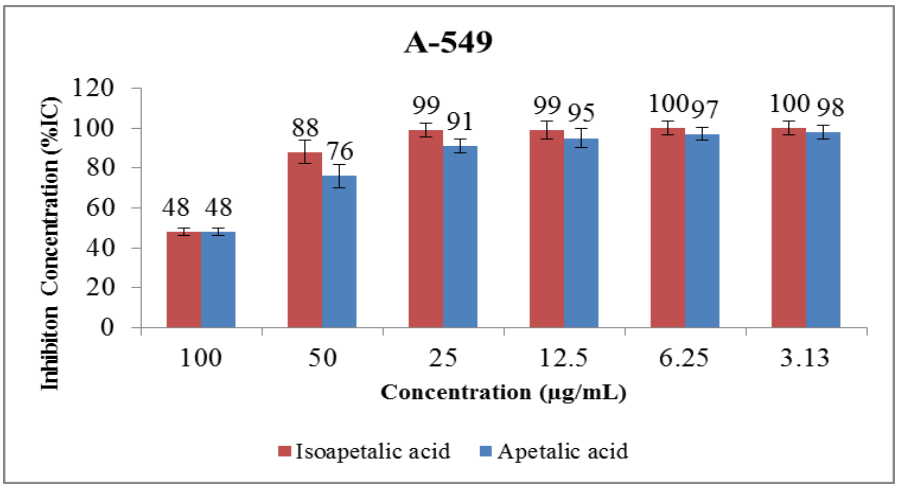
Three replicates of each sample were used for statistical analysis with values reported as mean ± SD. Standard curves were generated and calculation of the 50% inhibitory concentration (IC_{50}) values was

performed using GraphPad Prism for Windows (version 5.02) software. The Student's *t*-test was carried out using SPSS (version 22) software to study the comparison between treatment of samples and untreated control. A value of $p < 0.05$ was considered significantly different**.**

Bar Graph on Cytotoxic Activity of Isoapetalic acid (1) and Apetalic acid (2) against A-549 and MCF-7 cell lines at six different concentrations

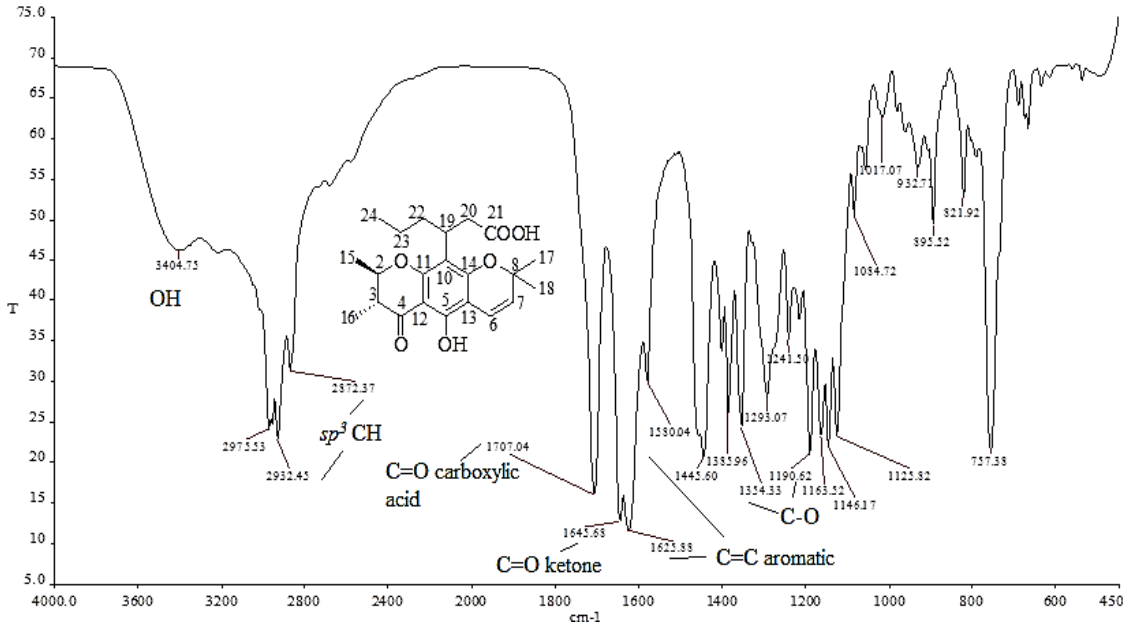


Percentage of Inhibition Concentration (%IC) of Isoapetalic acid **(1)** and Apetalic acid **(2)** against MCF-7

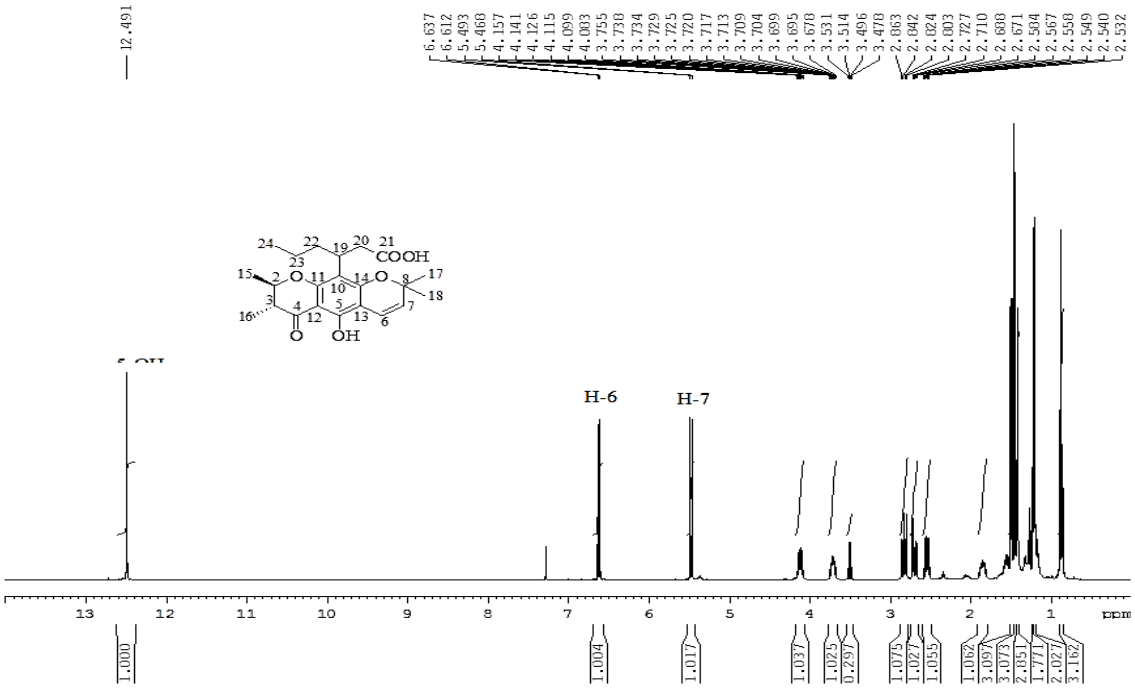


Percentage of Inhibition Concentration (%IC) of Isoapetalic acid **(1)** and Apetalic acid **(2)** against A-549

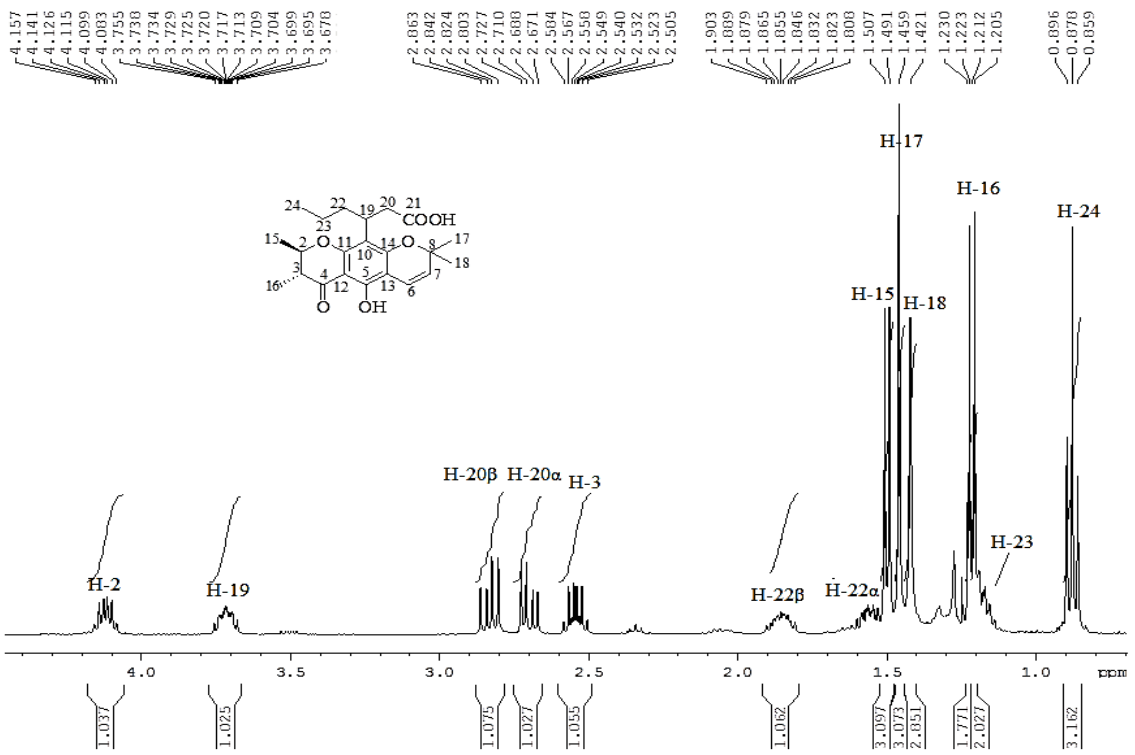
Isoapetalic acid **(1)**: Pale yellow gum; R_f 0.38 (*n*-Hex:Et_2O, 1:1); $[\alpha]_D^{25}$ -196.7º (*c* 0.033, $CHCl_3$); IR (NaCl disc, $CHCl_3$) υ_{max} cm^{-1}: 3405 (OH), 2975 and 2932 (sp^3 CH), 1707 (C=O acid), 1646 (chelate C=O ketone), 1626 and 1580 (C=C aromatic), 1354 and 1190 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* = 7.2 Hz, H-24), 1.20 (2H, m, H-23), 1.22 (3H, d, *J* = 7.2 Hz, H-16), 1.42 (3H, s, H-18), 1.46 (3H, s, H-17), 1.50 (3H, d, *J* = 6.4 Hz, H-15), 1.58 (1H, m, H-22α), 1.86 (1H, m, H-22β), 2.55 (1H, dq, *J* = 10.8 and 7.2 Hz, H-3), 2.68 (1H, dd, *J* = 15.6 and 6.8 Hz, H-20α), 2.83 (1H, dd, *J* = 15.2 and 8.4 Hz, H-20β), 3.71 (1H, m, H-19), 4.12 (1H, dq, *J* = 10.8 and 6.4 Hz, H-2), 5.48 (1H, d, *J* = 10.0 Hz, H-7), 6.62 (1H, d, *J* = 10.0 Hz, H-6) and 12.49 (1H, s, 5-OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.5 (C-16), 14.1 (C-24), 19.5 (C-15), 20.9 (C-23), 28.2 (C-17), 28.4 (C-18), 30.4 (C-19), 35.5 (C-22), 38.6 (C-20), 45.8 (C-3), 78.1 (C-8), 78.9 (C-2), 101.9 (C-12), 102.6 (C-13), 109.0 (C-10), 115.7 (C-6), 125.6 (C-7), 157.0 (C-5), 159.9 (C-11 and C-14), 179.0 (C-21) and 199.4 (C-4); EIMS (% rel int): *m/z* 388 (12), $[M]^+$ ($C_{22}H_{28}O_6$), 373 (100), 329 (5).



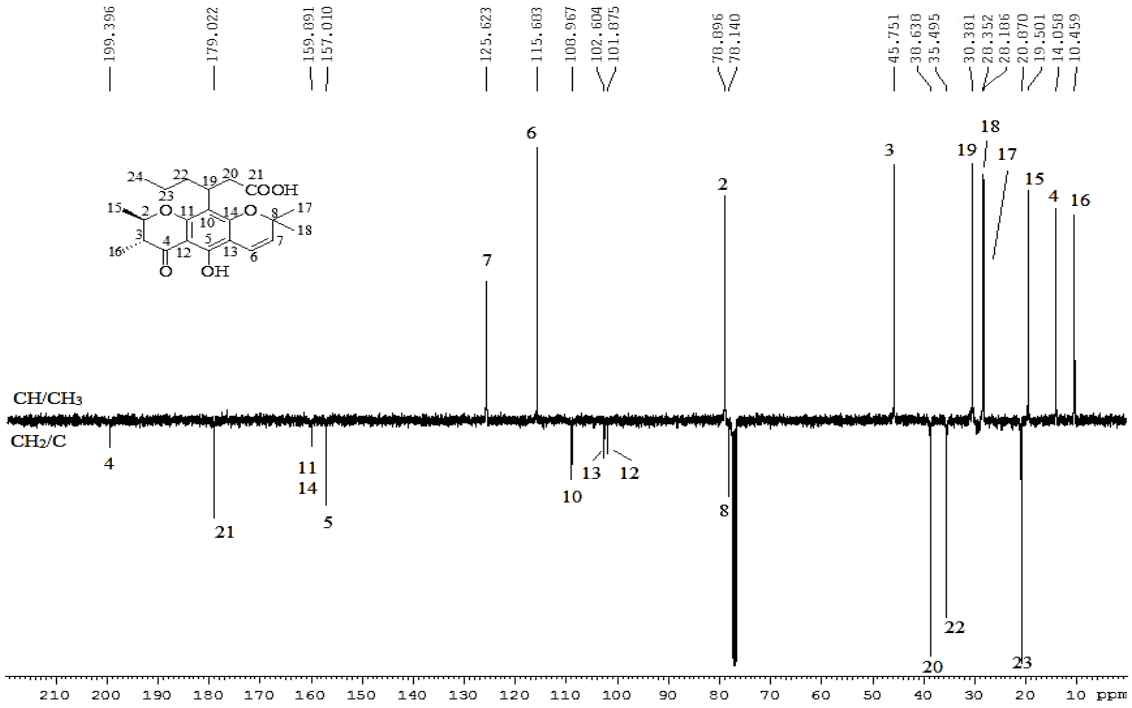
S1: IR spectrum of Isoapetalic acid **(1)**



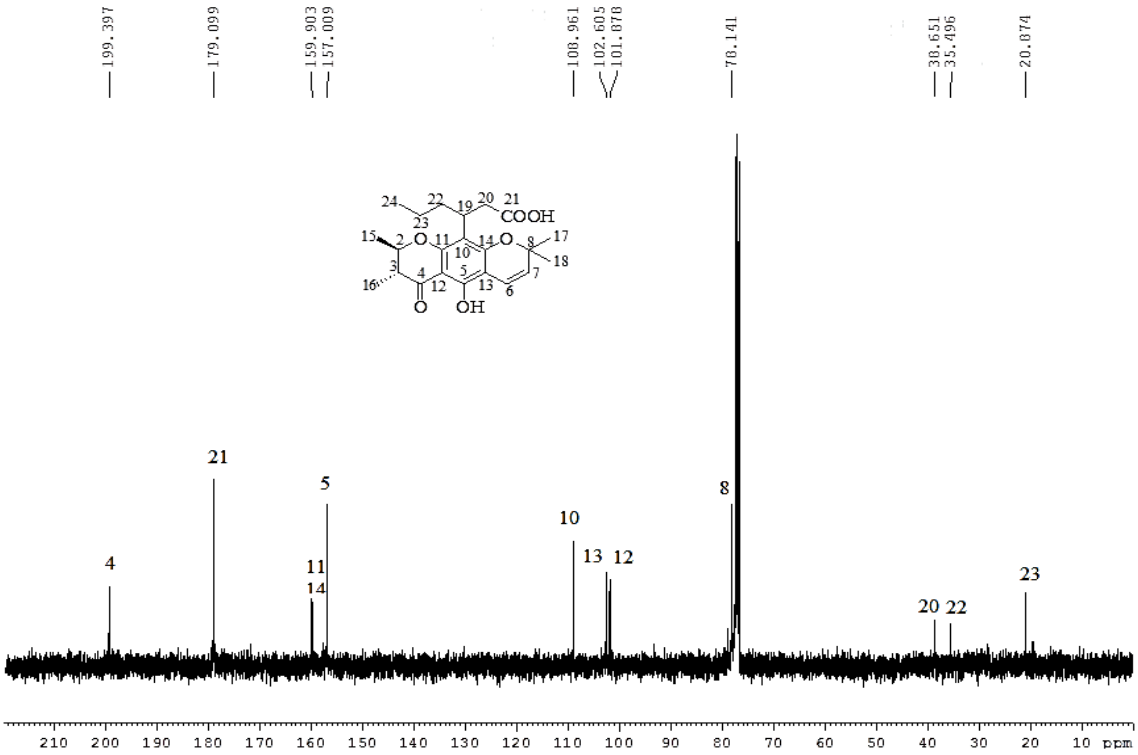
S2: ^{1}H NMR spectrum of Isoapetalic acid **(1)**



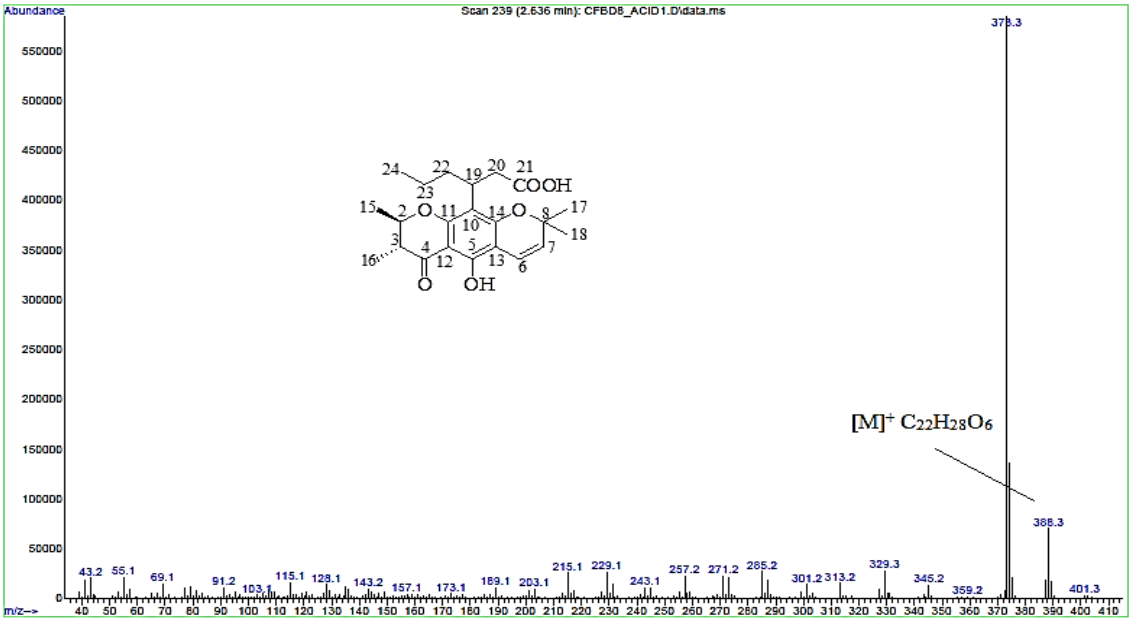
S3: ^{1}H NMR spectrum of Isoapetalic acid **(1)** (Expansion)



S4: DEPTQ spectrum of Isoapetalic acid **(1)**

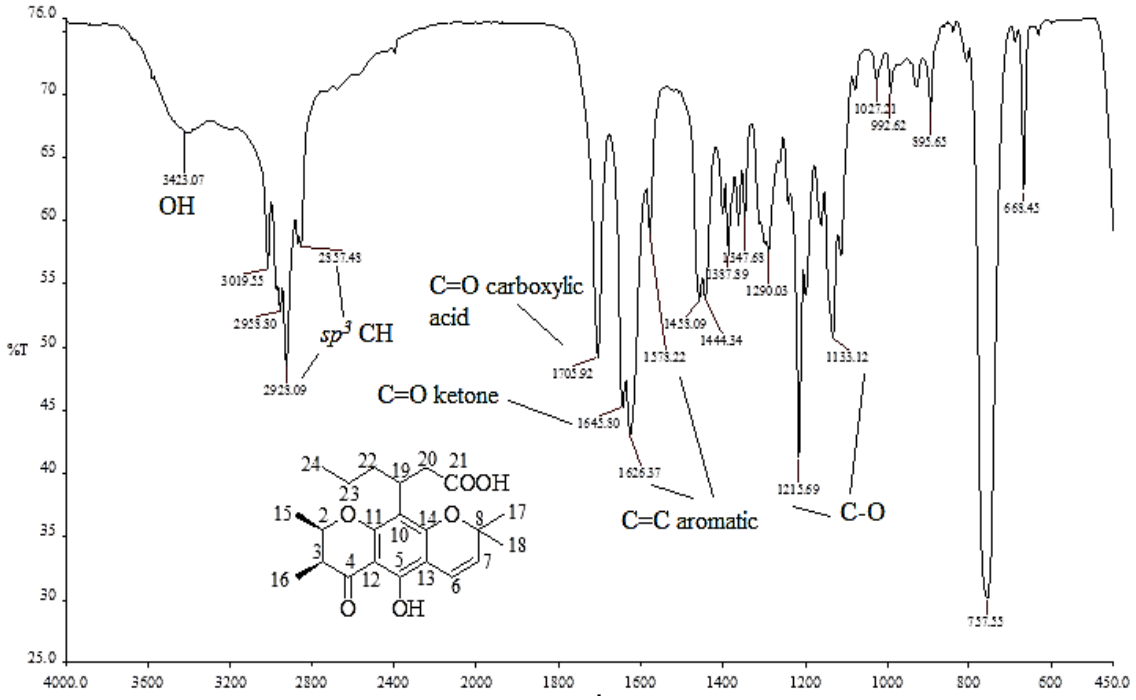


S5: DEPTQ_Q spectrum of Isoapetalic acid **(1)**

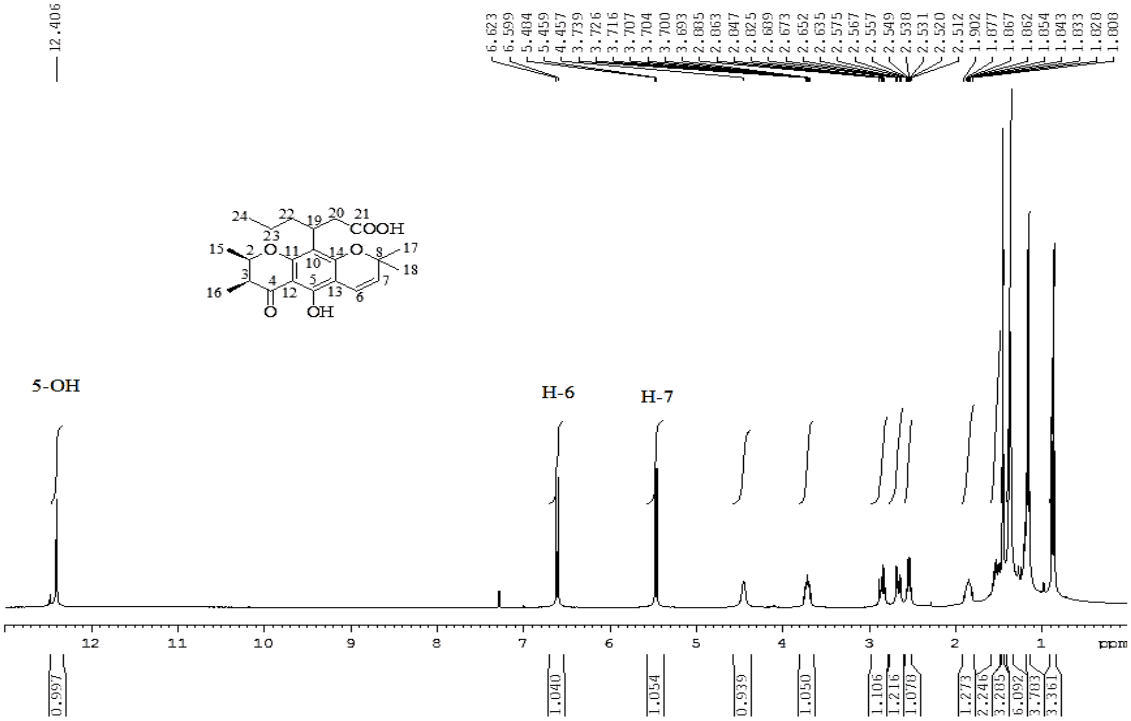


S6: EIMS spectrum of Isoapetalic acid **(1)**

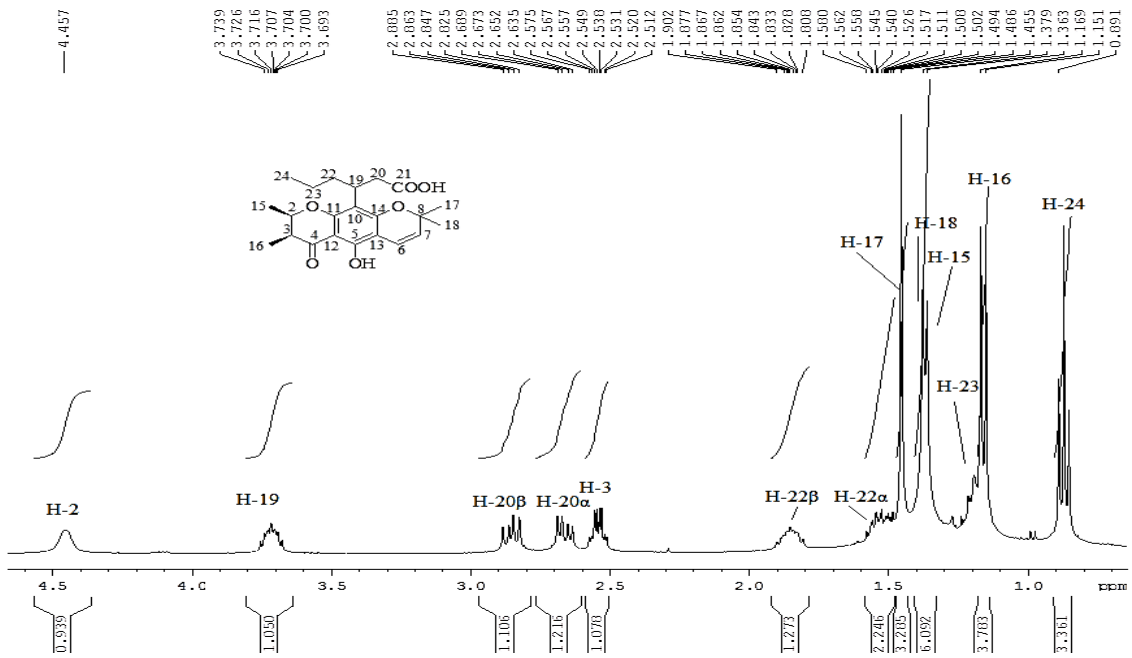
Apetalic acid **(2)**: Yellow gum; R_f 0.25 (*n*-Hex:Et_2O, 1:1); $[\alpha]_D^{25}$ -73.4º (*c* 0.033, $CHCl_3$); IR (NaCl disc, $CHCl_3$) υ_{max} cm^{-1}: 3423 (OH), 2928 and 2857 (sp^3 CH), 1705 (C=O acid), 1646 (chelate C=O ketone), 1626 and 1578 (C=C aromatic), 1215 and 1133 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (3H, t, *J* = 7.2 Hz, H-24), 1.16 (3H, d, *J* = 7.2 Hz, H-16), 1.20 (2H, m, H-23), 1.37 (3H, d, *J* = 6.4 Hz, H-15), 1.38 (3H, s, H-18), 1.46 (3H, s, H-17), 1.58 (1H, m, H-22α), 1.86 (1H, m, H-22β), 2.55 (1H, qd, *J* = 7.2 and 3.2 Hz, H-3), 2.66 (1H, dd, *J* = 15.6 and 6.8 Hz, H-20α), 2.85 (1H, dd, *J* = 15.2 and 8.4 Hz, H-20β), 3.71 (1H, m, H-19), 4.46 (1H, br s, H-2), 5.47 (1H, d, *J* = 10.0 Hz, H-7), 6.61 (1H, d, *J* = 10.0 Hz, H-6) and 12.41 (1H, s, 5-OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.3 (C-16), 14.0 (C-24), 16.3 (C-15), 20.8 (C-23), 28.1 (C-17), 28.4 (C-18), 30.5 (C-19), 35.5 (C-22), 38.6 (C-20), 44.2 (C-3), 76.1 (C-2), 78.2 (C-8), 101.2 (C-12), 102.6 (C-13), 108.7 (C-10), 115.6 (C-6), 125.7 (C-7), 157.3 (C-5), 159.9 (C-11 and C-14), 179.4 (C-21) and 201.3 (C-4); EIMS (% rel int): *m/z* 388 (12), $[M]^+$ ($C_{22}H_{28}O_6$), 373 (100), 329 (5).



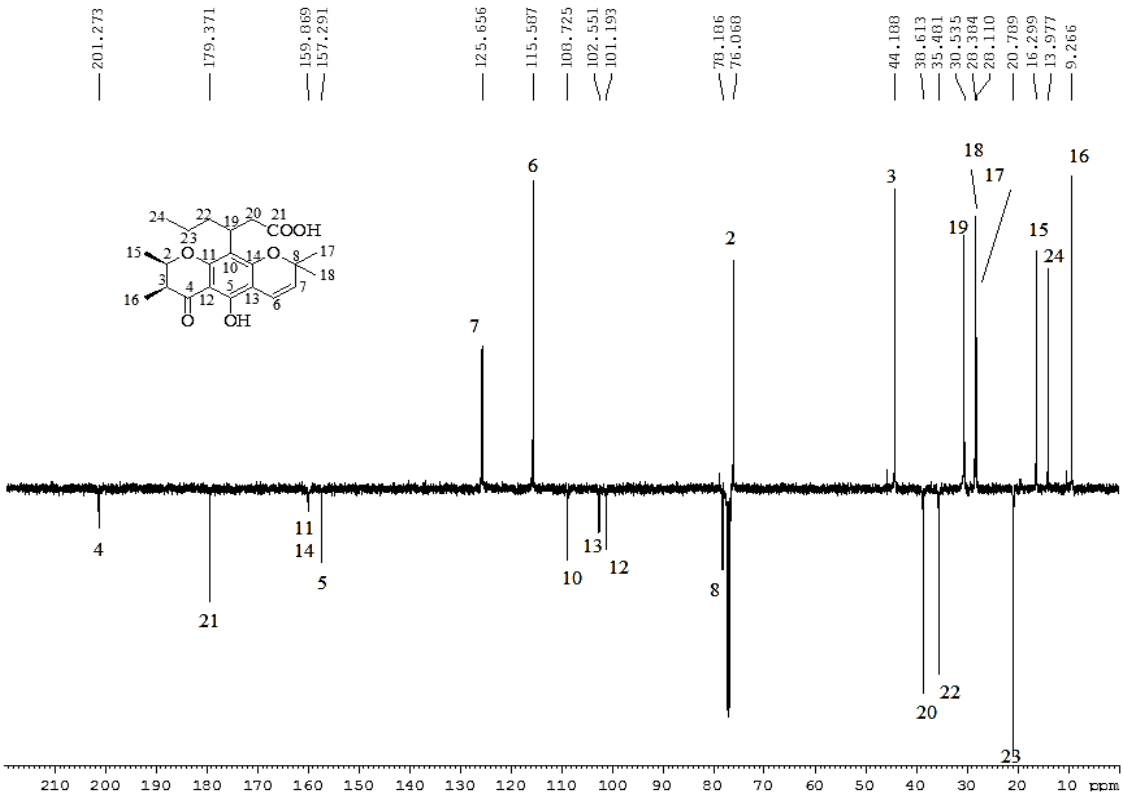
S7: IR spectrum of Apetalic acid **(2)**



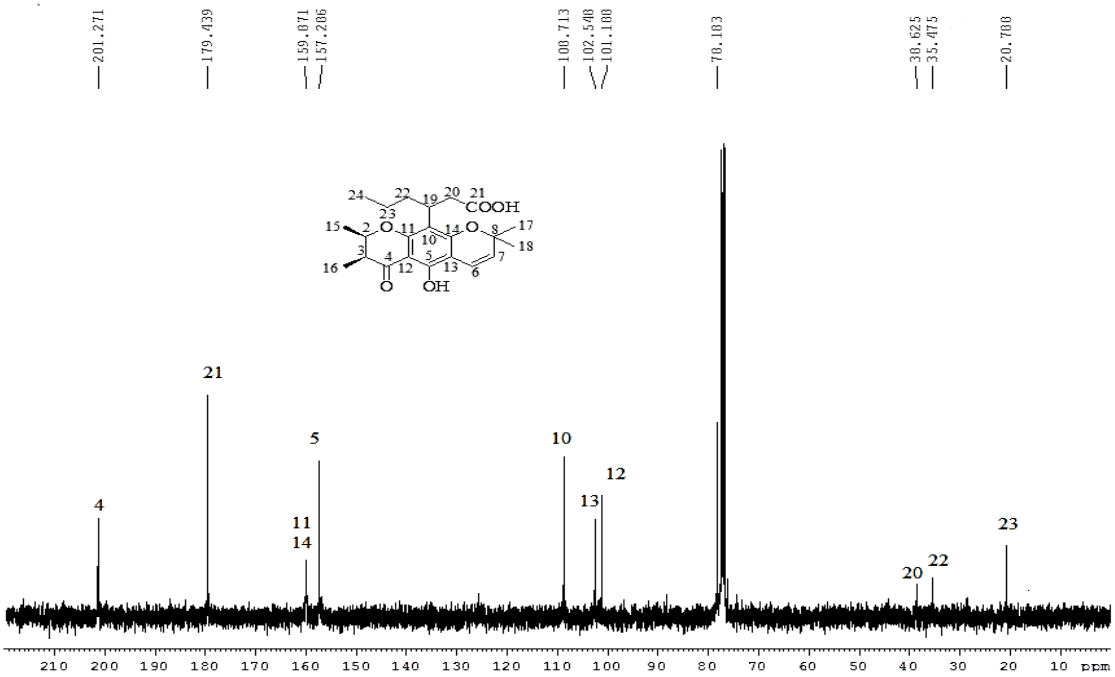
S8: ^{1}H NMR spectrum of Apetalic acid **(2)**



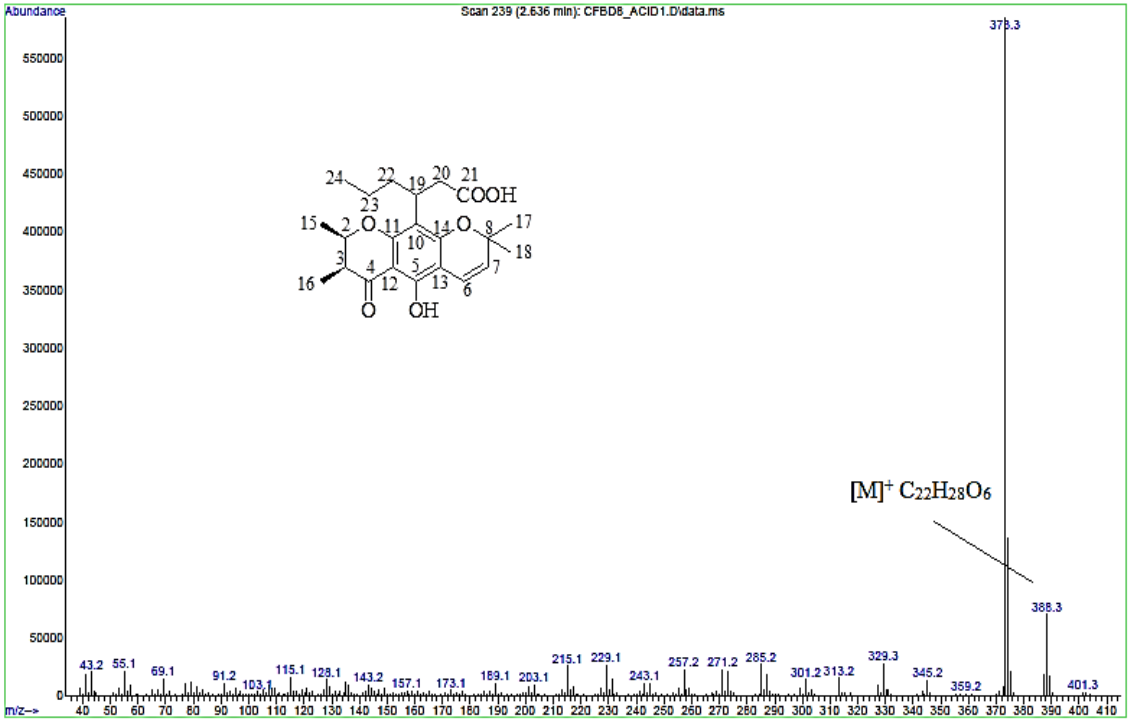
S9: ^{1}H NMR spectrum of Apetalic acid **(2)** (Expansion)



S10: DEPTQ spectrum of Apetalic acid **(2)**

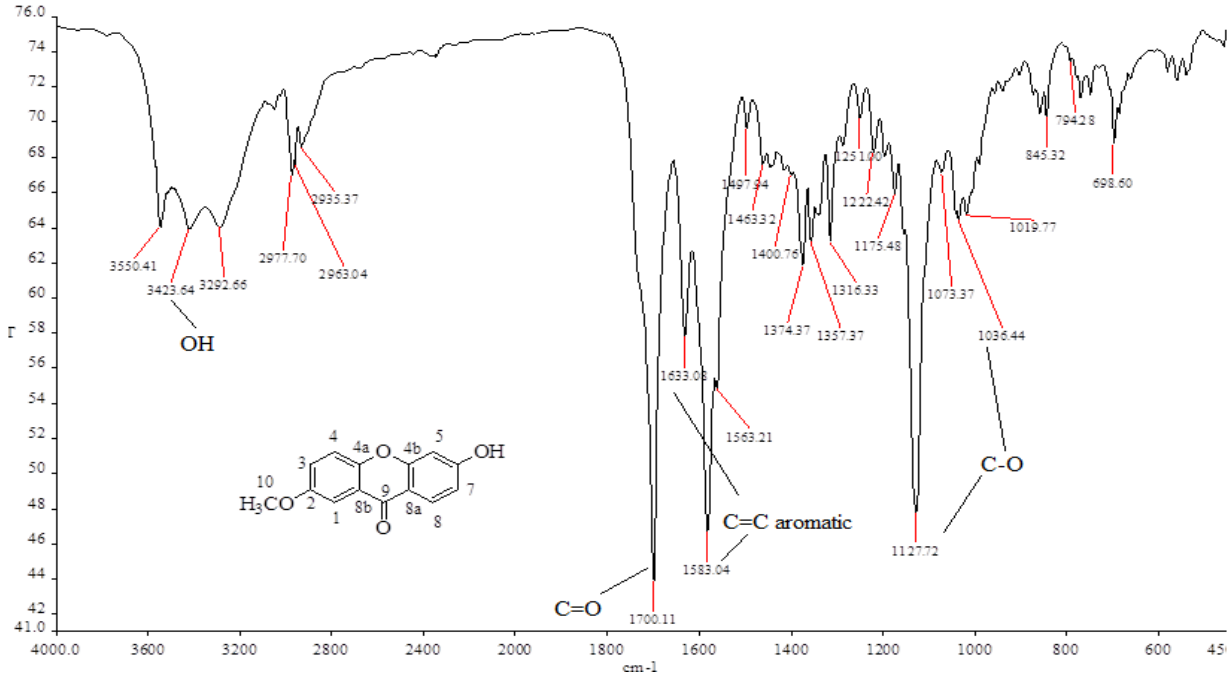


S11: DEPTQ_Q spectrum of Apetalic acid **(2)**

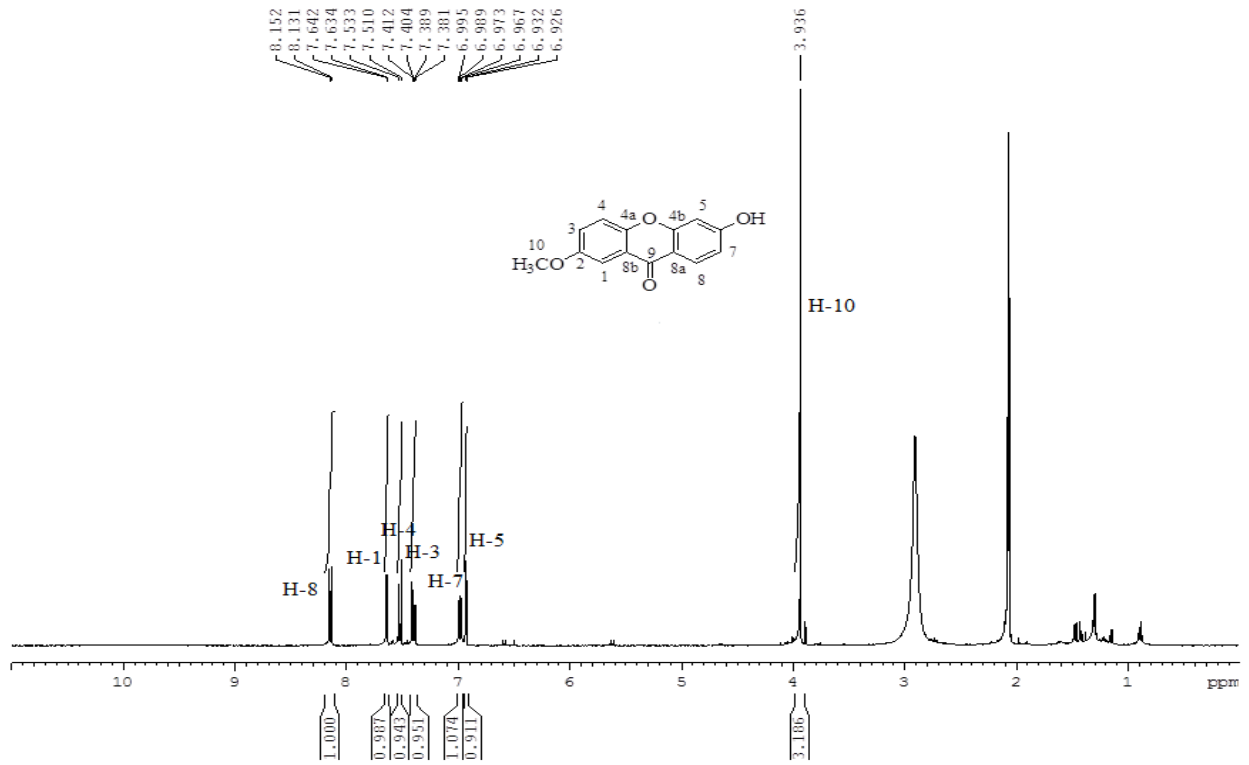


S12: EIMS spectrum of Apetalic acid **(2)**

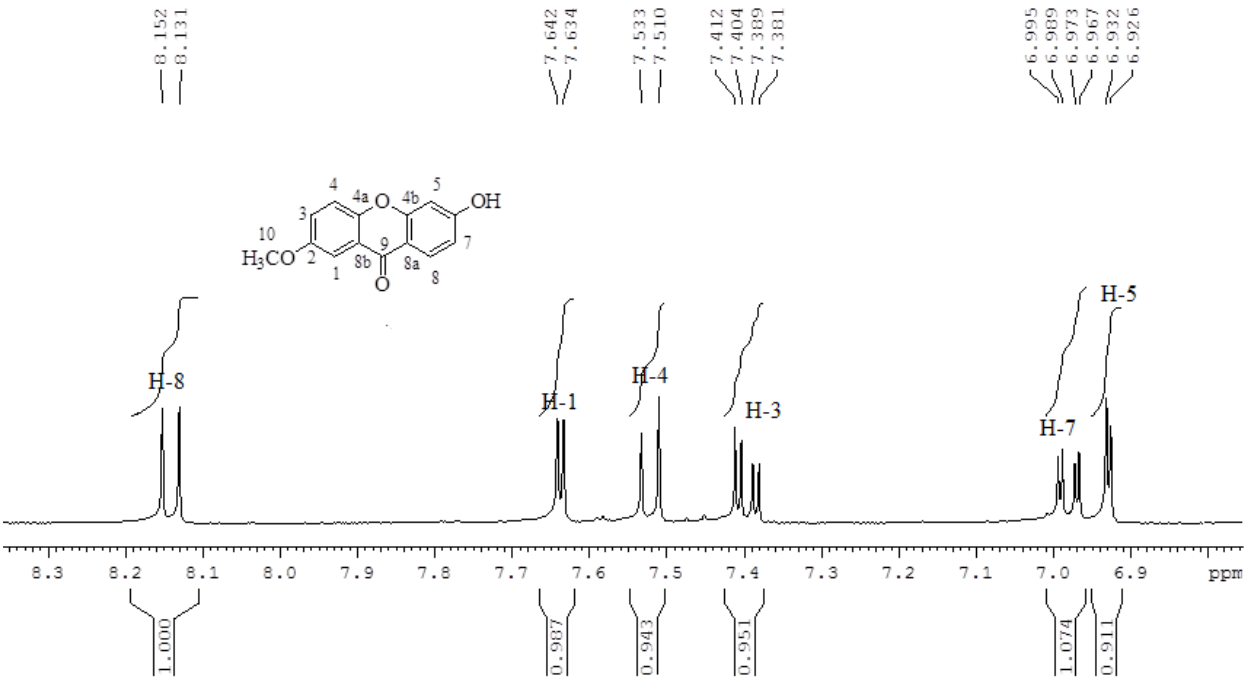
6-Hydroxy-2-methoxyxanthone **(3)**: Pale yellow solid; R_f 0.45 (*n*-Hex:EtOAc, 1:1); m.p 263 – 265ºC; IR (KBr pellet) υ_{max} cm^{-1}: 3423 (OH), 1700 (C=O ketone), 1633 and 1583 (C=C aromatic), 1127 and 1036 (C-O); ^{1}H NMR (400 MHz, CD_3COCD_3): δ 3.94 (3H, s, 10-OCH_3), 6.93 (1H, d, *J* = 2.4 Hz, H-5), 6.98 (1H, dd, *J* = 8.8 and 2.4 Hz, H-7), 7.40 (1H, dd, *J* = 9.2 and 3.2 Hz, H-3), 7.53 (1H, d, *J* = 9.2 Hz, H-4), 7.64 (1H, d, *J* = 3.2 Hz, H-1), 8.14 (1H, d, *J* = 8.8 Hz, H-8) and 9.93 (1H, s, 6-OH); ^{13}C NMR (100 MHz, CD_3COCD_3): δ 55.3 (C-10), 102.1 (C-5), 106.1 (C-1), 113.8 (C-7), 114.4 (C-8a), 119.2 (C-4), 122.2 (C-8b), 123.5 (C-3), 128.1 (C-8), 150.7 (C-4a), 156.1 (C-2), 158.0 (C-4b), 163.7 (C-6) and 174.9 (C-9); EIMS (% rel int): *m/z* 242 (100), $[M]^+$ ($C_{14}H_{10}O_4$), 227 (24), 212 (31).



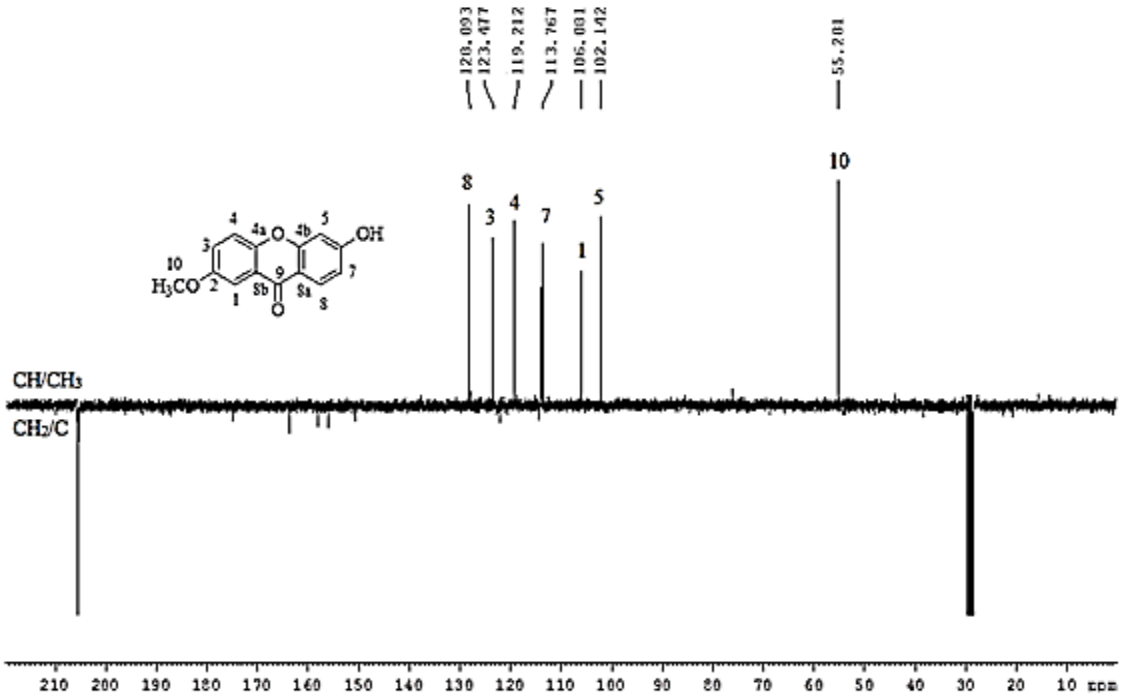
S13: IR spectrum of 6-Hydroxy-2-methoxyxanthone **(3)**



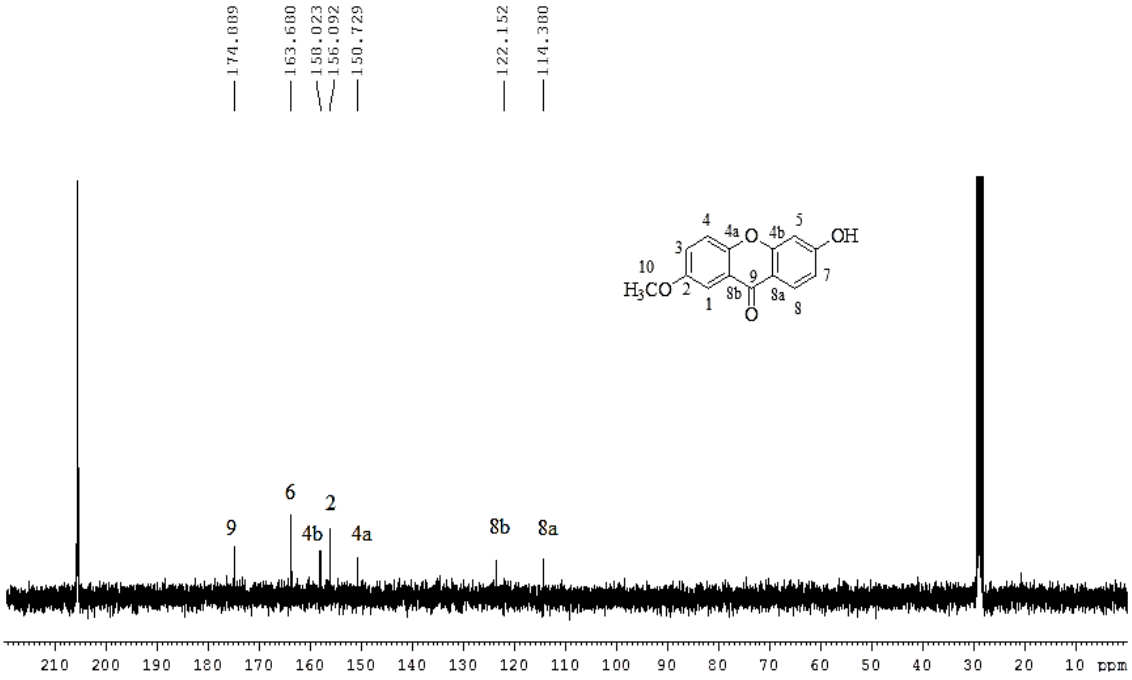
S14: [1]H NMR spectrum of 6-Hydroxy-2-methoxyxanthone **(3)**



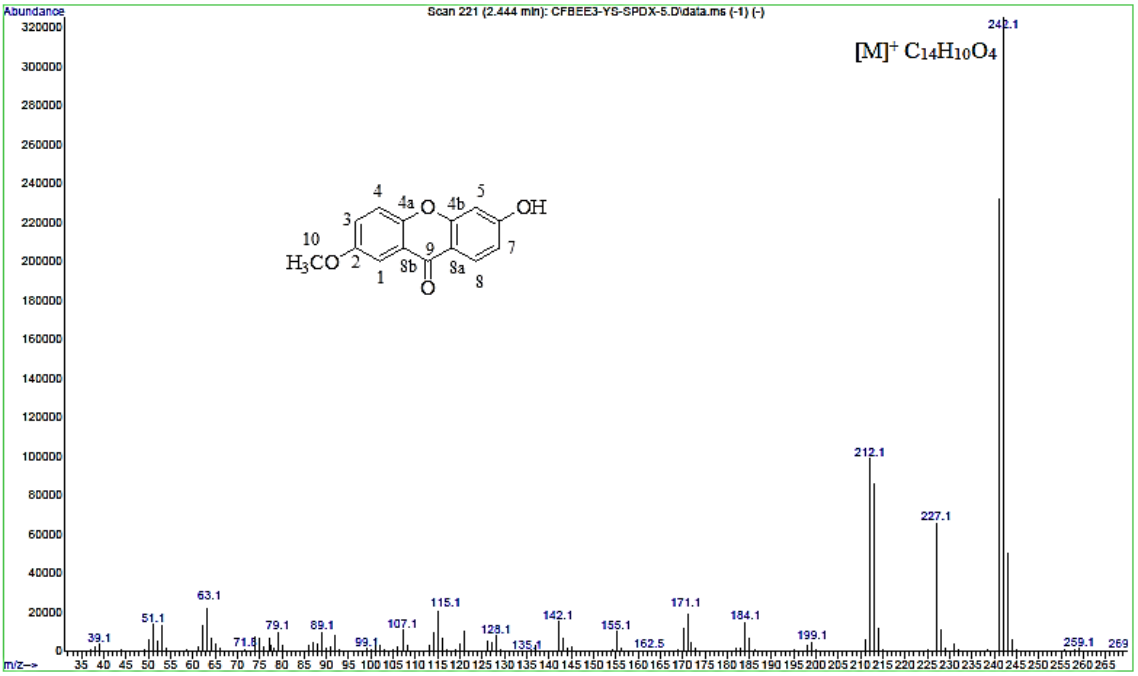
S15: [1]H NMR spectrum of 6-Hydroxy-2-methoxyxanthone **(3)** (Expansion)



S16: DEPTQ spectrum of 6-Hydroxy-2-methoxyxanthone **(3)**

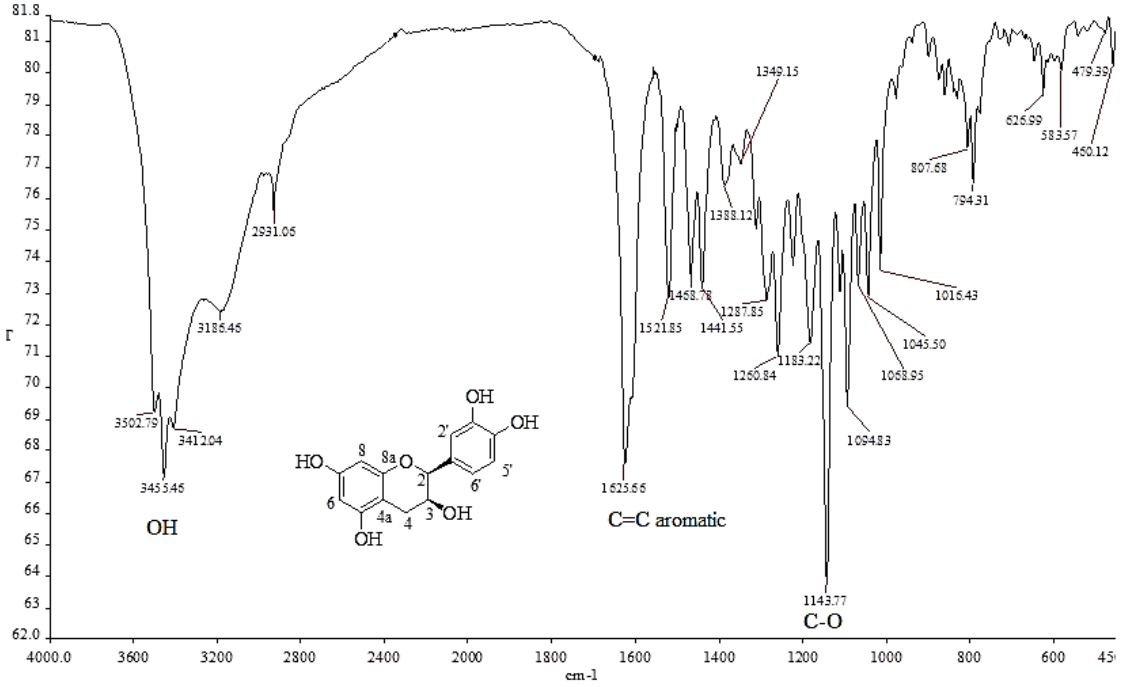


S17: DEPTQ_Q spectrum of 6-Hydroxy-2-methoxyxanthone **(3)**

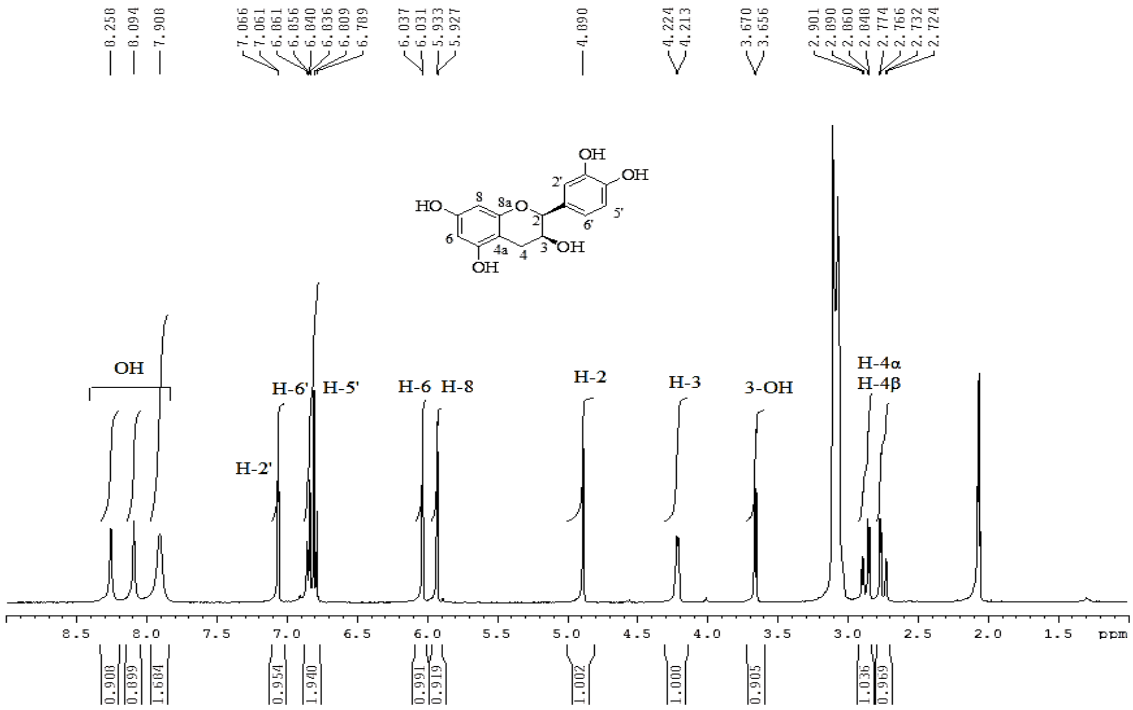


S18: EIMS spectrum of 6-Hydroxy-2-methoxyxanthone **(3)**

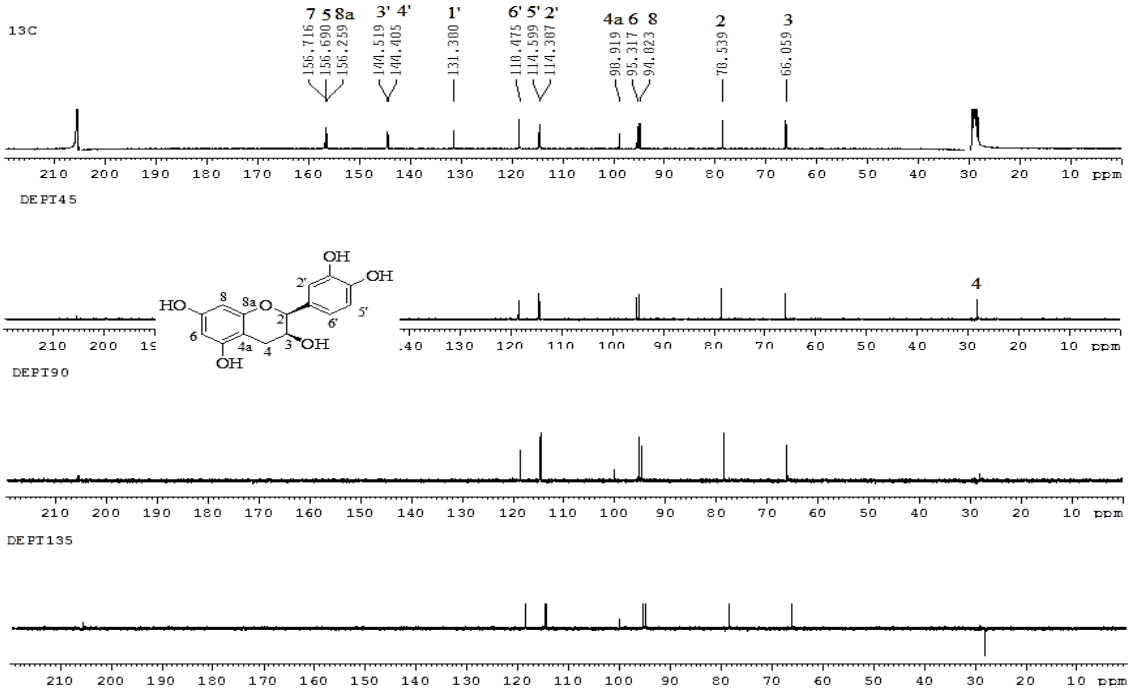
ent-Epicatechin **(4)**: Pale brown amorphous; R_f 0.25 (*n*-Hex:EtOAc, 1:1); m.p 235 – 236ºC; $[\alpha]_D^{25}$ +48.9º (*c* 0.067, MeOH); IR (KBr pellet) υ $_{max}$ cm^{-1}: 3455 (OH), 2931 (sp^3 CH), 1625 (C=C aromatic), 1143 and 1016 (C-O); ^{1}H NMR (400 MHz, CD_3COCD_3): δ 2.75 (1H, dd, *J* = 16.8 and 3.2 Hz, H-4α), 2.86 (1H, dd, *J* = 16.8 and 4.4 Hz, H-4β), 3.66 (1H, d, *J* = 5.6 Hz, 3-OH), 4.22 (1H, br s, H-3), 4.89 (1H, s, H-2), 5.93 (1H, d, *J* = 2.4 Hz, H-8), 6.03 (1H, d, *J* = 2.4 Hz, H-6), 6.79 (1H, d, *J* = 8.0 Hz, H-5'), 6.85 (1H, dd, *J* = 8.0 and 2.0 Hz, H-6'), 7.06 (1H, d, *J* = 2.0 Hz, H-2'), 7.91 (2H, br s, 3'-OH and 4'-OH), 8.09 (1H, br s, 7-OH) and 8.26 (1H, br s, 5-OH); ^{13}C NMR (100 MHz, CD_3COCD_3): δ 28.2 (C-4α and C-4β), 66.0 (C-3), 78.5 (C-2), 94.7 (C-8), 95.3 (C-6), 98.9 (C-4a), 114.4 (C-2'), 114.6 (C-5'), 118.4 (C-6'), 131.3 (C-1'), 144.4 (C-4'), 144.5 (C-3'), 156.2 (C-8a), 156.6 (C-5) and 156.7 (C-7); EIMS (% rel int): *m/z* 291 (4) $[M+H]^+$, *m/z* 290 (22), $[M]^+$ ($C_{15}H_{14}O_6$), 152 (40), 139 (100), 123 (46).



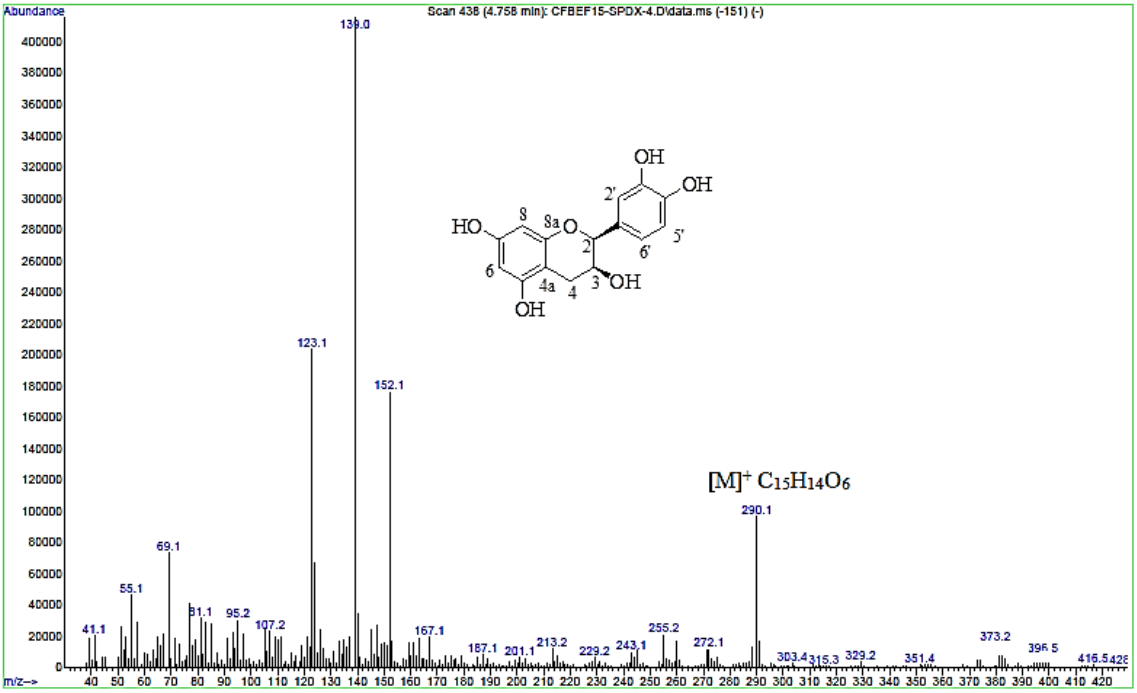
S19: IR spectrum of *ent*-Epicatechin **(4)**



S20: ^{1}H NMR spectrum of *ent*-Epicatechin **(4)**

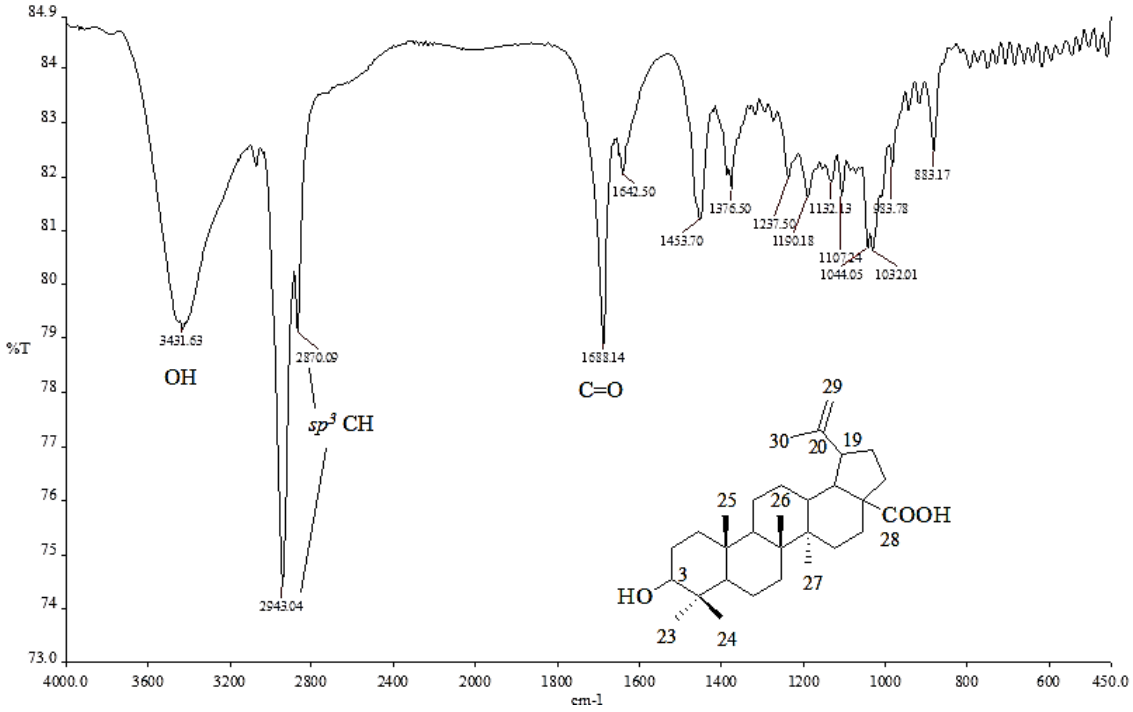


S21: ^{13}C/DEPT spectra of *ent*-Epicatechin **(4)**

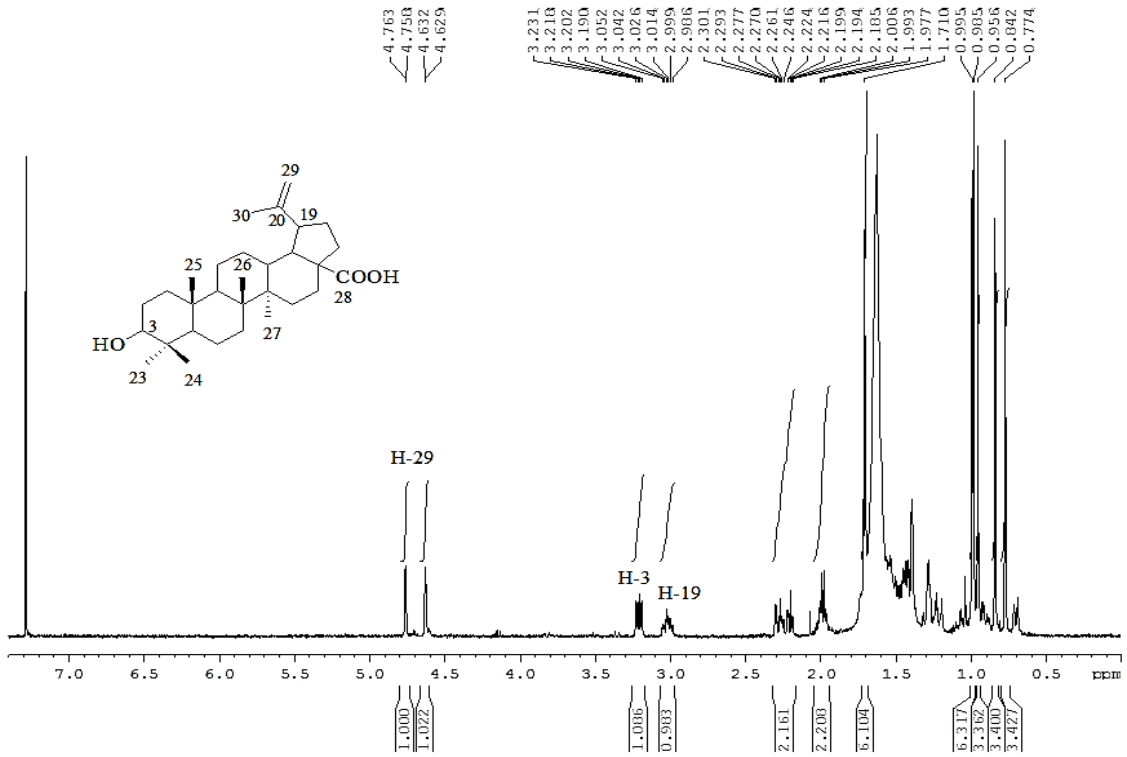


S22: EIMS spectrum of *ent*-Epicatechin **(4)**

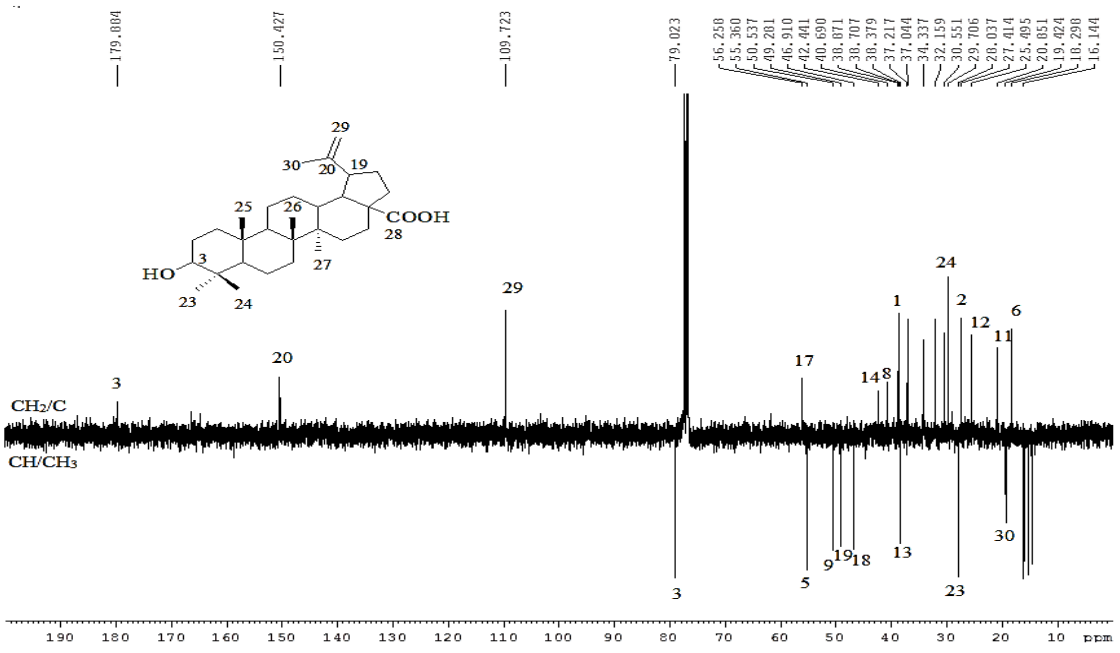
Betulinic acid **(5)**: White solid; R_f 0.55 (*n*-Hex: EtOAc, 3:2); m.p 311 – 313ºC; IR (KBr pellet) υ_{max} cm^{-1}: 3431 (OH), 2943 and 2870 (sp^3 CH) and 1688 (C=O carboxylic acid); ^1H NMR (400 MHz, $CDCl_3$): δ 0.77 (3H, s, H-24), 0.84 (3H, s, H-25), 0.96 (3H, s, H-27), 0.98 (3H, s, H-23), 0.99 (3H, s, H-26), 1.71 (3H, s, H-30), 3.00 (1H, m, H-19), 3.21 (1H, dd, *J* = 4.8 and 11.2 Hz, H-3), 4.76 (1H, br s, H_a-29), 4.73 (1H, br s, H_b-29); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.7 (C-27), 15.4 (C-24), 16.0 (C-25), 16.1 (C-26), 18.3 (C-6), 19.4 (C-30), 20.9 (C-11), 25.5 (C-12), 27.4 (C-2), 28.0 (C-23), 29.7 (C-21), 30.5 (C-15), 32.2 (C-16), 34.3 (C-7), 37.0 (C-22), 37.2 (C-10), 38.4 (C-13), 38.7 (C-1), 38.9 (C-4), 40.7 (C-8), 42.4 (C-14), 46.9 (C-18), 49.2 (C-19), 50.5 (C-9), 55.4 (C-5), 56.3 (C-17), 79.0 (C-3), 109.7 (C-29), 150.4 (C-20), 179.9 (C-28); EIMS (% rel int): *m/z* 456 (7) $[M]^+$ ($C_{30}H_{48}O_3$), 248 (24), 203 (45), 189 (100).



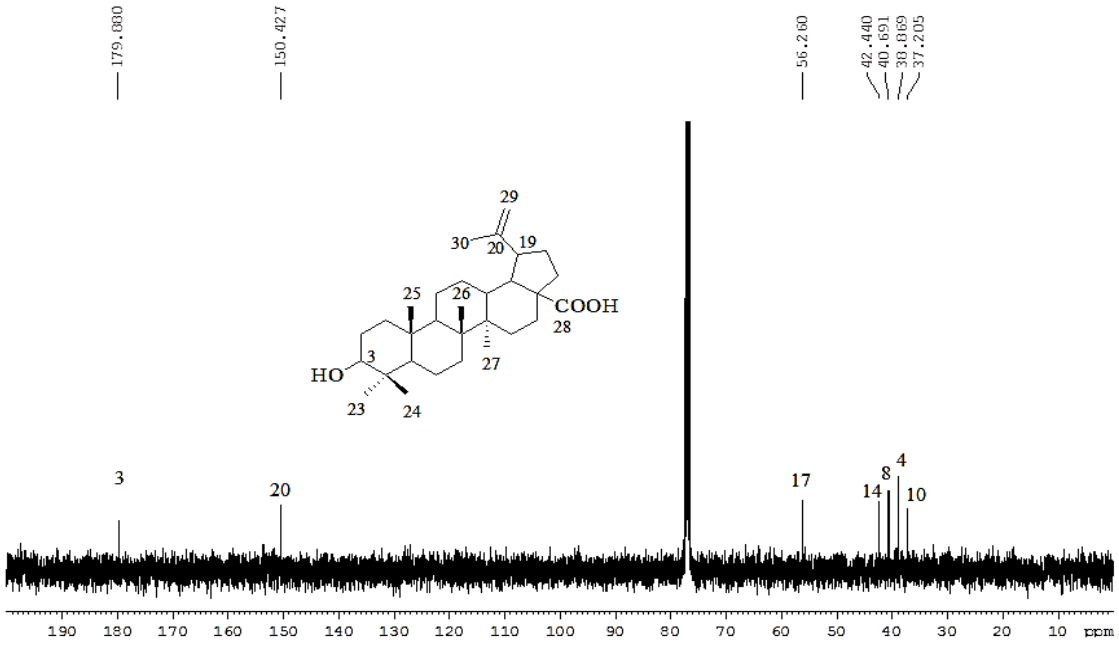
S23: IR spectrum of Betulinic acid **(5)**



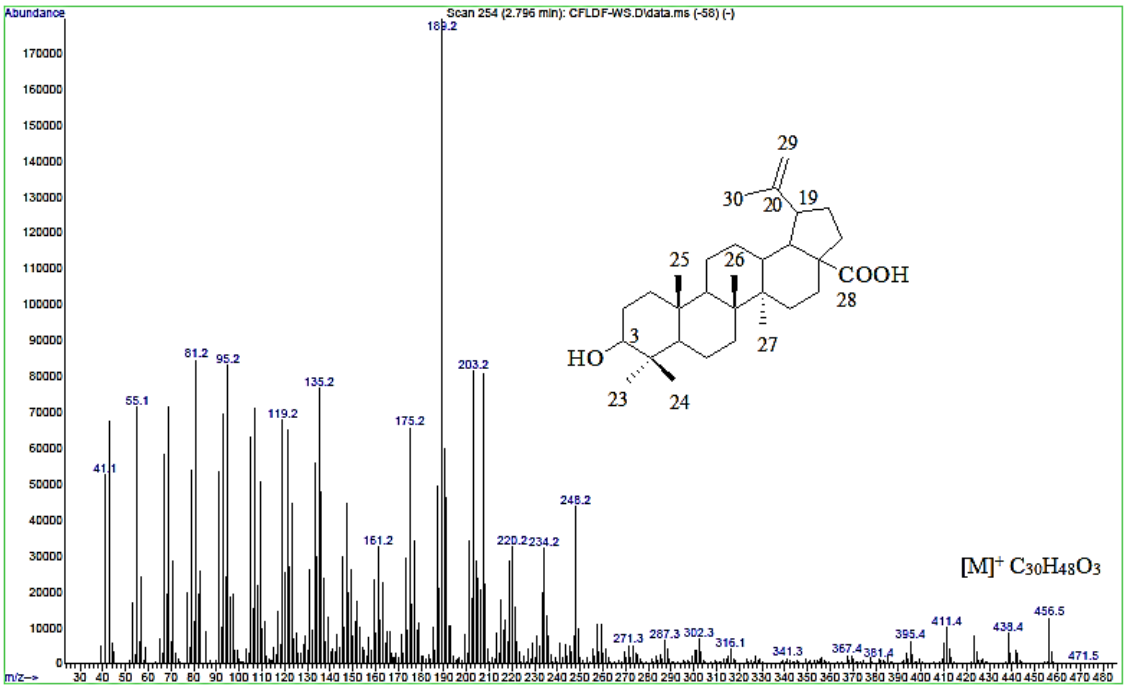
S24: ^{1}H NMR spectrum of Betulinic acid **(5)**



S25: DEPTQ spectrum of Betulinic acid **(5)**

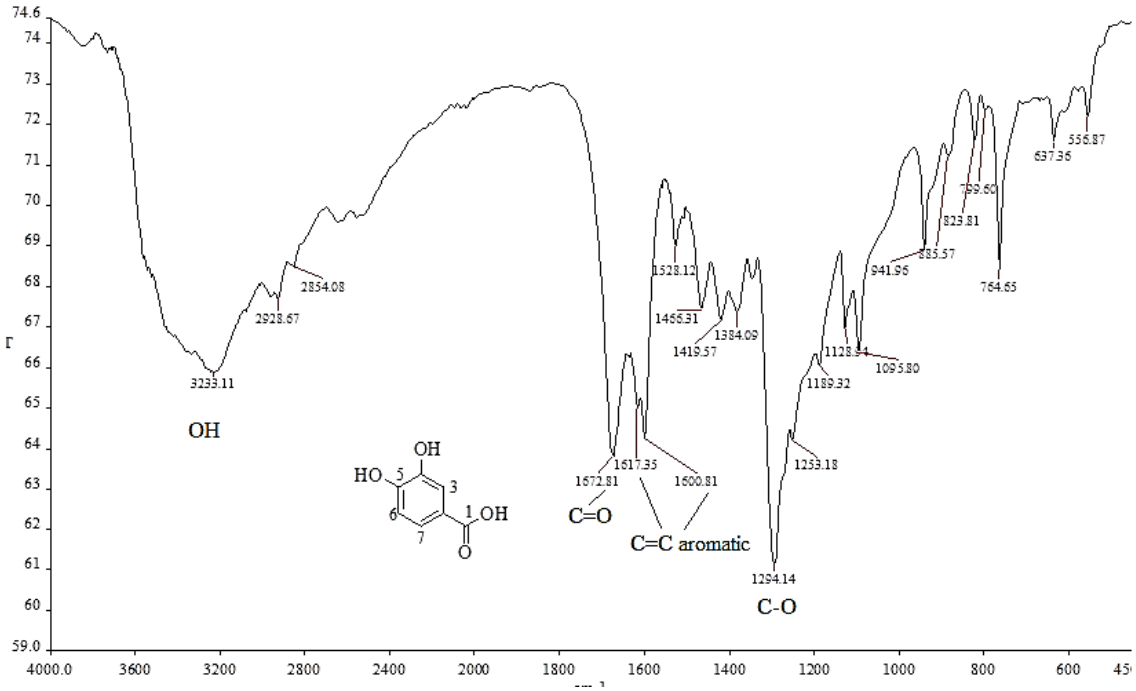


S26: DEPTQ_Q spectrum of Betulinic acid **(5)**

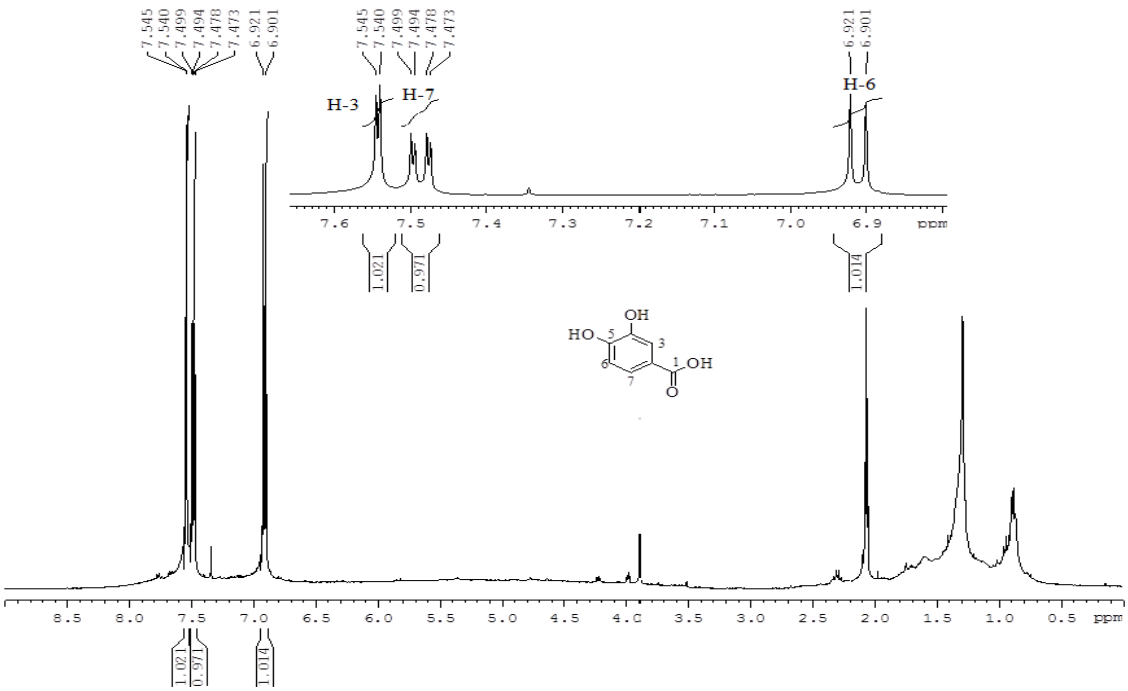


S27: EIMS spectrum of Betulinic acid **(5)**

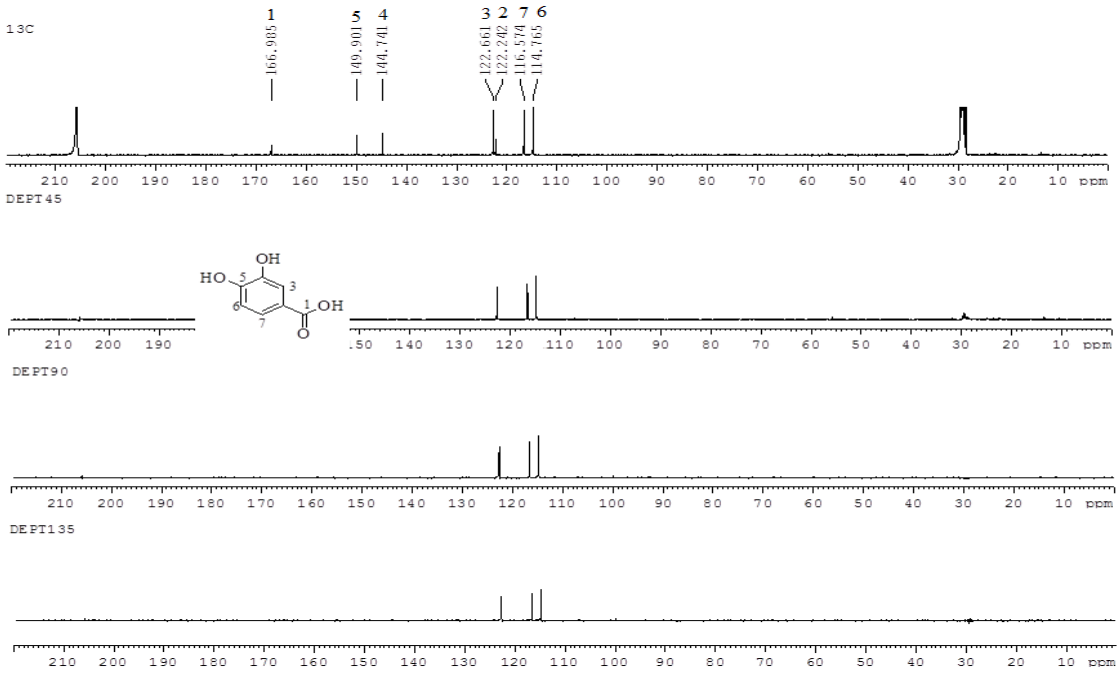
Protocatechuic acid **(6)**: Yellow needle; R_f 0.33 (*n*-Hex:EtOAc, 1:4); m.p 197 – 198ºC; IR (KBr pellet) υ_{max} cm^{-1}: 3244 (OH), 1673 (conjugated C=O carboxylic acid), 1617 and 1600 (C=C aromatic) and 1294 and 1095 (C-O); ^1H NMR (400 MHz, CD_3COCD_3): δ 6.91 (1H, d, *J* = 8.0 Hz, H-6), 7.49 (1H, dd, *J* = 8.0 and 2.0 Hz, H-7), 7.54 (1H, d, *J* = 2.0 Hz, H-3); ^{13}C NMR (100 MHz, CD_3COCD_3): δ 114.8 (C-6), 116.6 (C-7), 122.2 (C-2), 122.7 (C-3), 144.7 (C-4), 149.9 (C-5) and 166.9 (C-1); EIMS (% rel int): *m/z* 154 (84) $[M]^+$ ($C_7H_6O_4$), 137 (100), 109 (24).



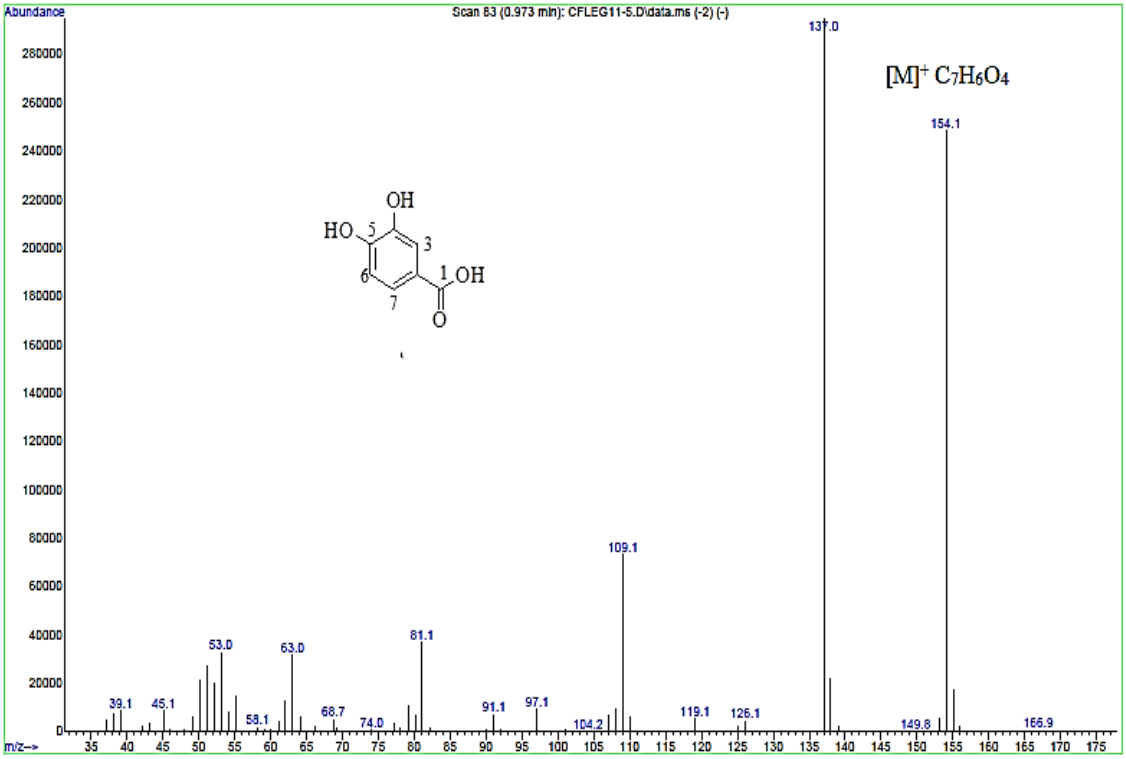
S28: IR spectrum of Protocatechuic acid **(6)**



S29: ^{1}H NMR of Protocatechuic acid **(6)**

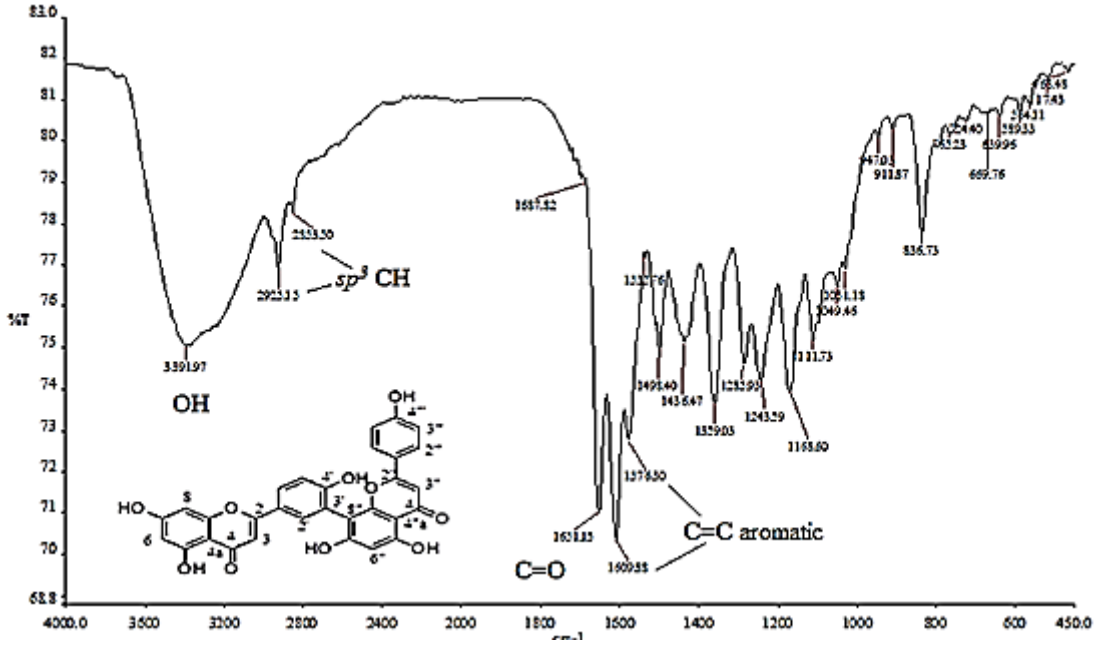


S30: ^{13}C/DEPT spectra of Protocatechuic acid **(6)**

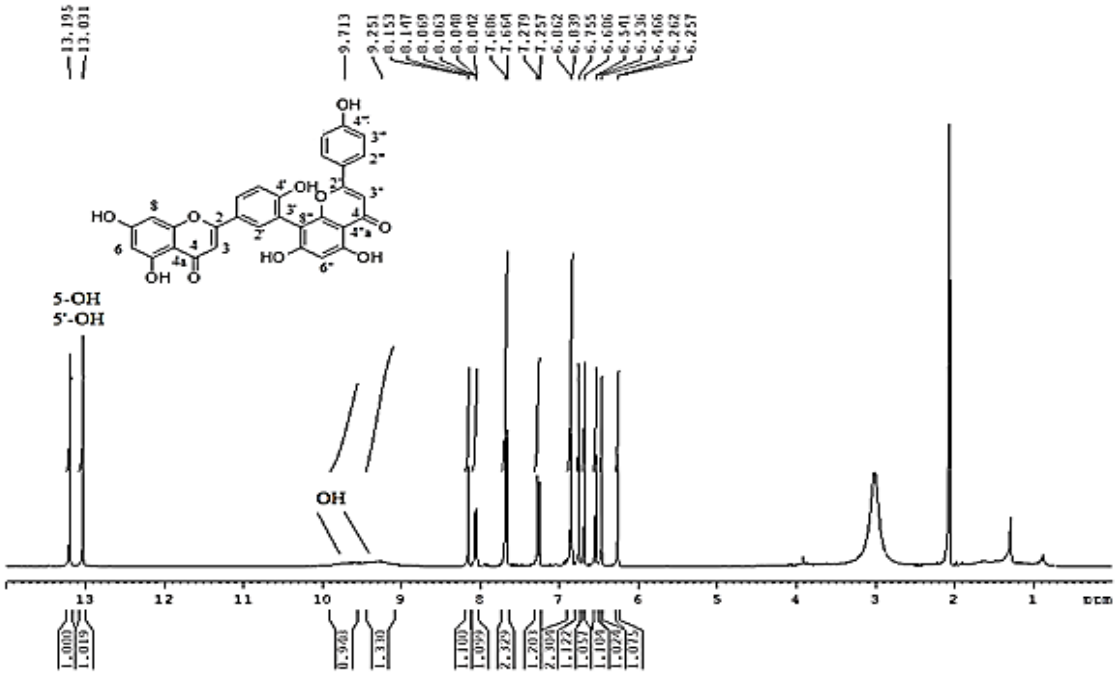


S31: EIMS spectrum of Protocatechuic acid **(6)**

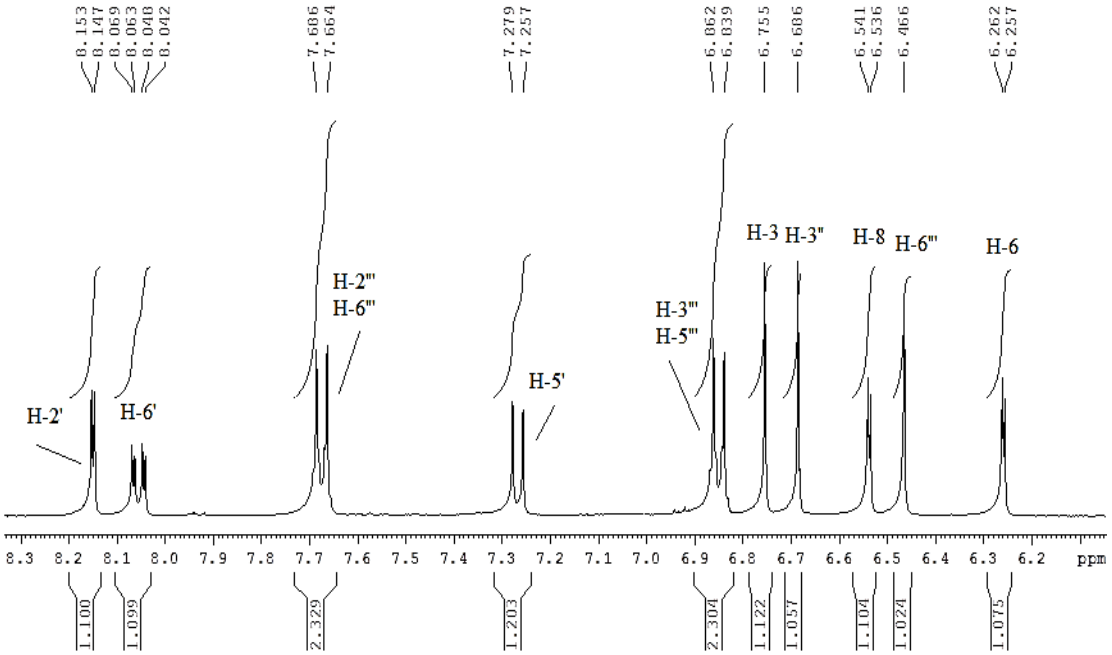
Amentoflavone **(7)**: Yellow amorphous; R_f 0.50 (*n*-Hex:EtOAc, 1:4); m.p 254 – 255ºC; IR (KBr pellet) υ_{max} cm^{-1}: 3392 (OH), 1651 (chelate C=O ketone), 1609 and 1576 (C=C aromatic) and 1168 and 1111 (C-O); ^{1}H NMR (400 MHz, CD_3COCD_3): δ 6.26 (1H, d, *J* = 2.4 Hz, H-6), 6.47 (1H, s, H-6”), 6.54 (1H, d, *J* = 2.0 Hz, H-8), 6.70 (1H, s, H-3”), 6.76 (1H, s, H-3), 6.86 (2H, d, *J* = 8.8 Hz, H-3”’ and H-5”’), 7.27 (1H, d, *J* = 8.8 Hz, H-5’), 7.69 (2H, d, *J* = 8.8 Hz, H-2”’ and H-6”’), 8.07 (1H, dd, *J* = 8.8 and 2.4 Hz, H-6’), 8.16 (1H, d, *J* = 2.4 Hz, H-2’), 9.26 (1H, br s, 4’-OH), 9.73 (1H, br s, 4”’-OH), 13.05 (1H, s, 5-OH) and 13.21 (1H, s, 5”-OH). ^{13}C NMR (100 MHz, CD_3COCD_3): δ 93.9 (C-8), 98.9 (C-6 and C-6”), 102.9 (C-3”), 103.4 (C-3), 103.5 (C-8”), 104.5 (C-4a), 104.6 (C-4”a), 115.9 (C-3”’ and C-5”’), 116.6 (C-5’), 119.9 (C-3’), 122.4 (C-1’), 122.5 (C-1”’), 127.9 (C-6’), 128.3 (C-2”’ and C-6”’), 131.7 (C-2’), 155.2 (C-8”a), 157.9 (C-8a), 159.4 (C-4’), 161.0 (C-5”), 161.7 (C-5), 161.9 (C-7”), 162.5 (C-4”’), 164.0 (C-2 and C-7), 164.2 (C-2”), 182.2 (C-4) and 182.6 (C-4”); ESIMS (% rel int): *m/z* 537 (100) $[M-H]^+$ ($C_{30}H_{18}O_{10}$).



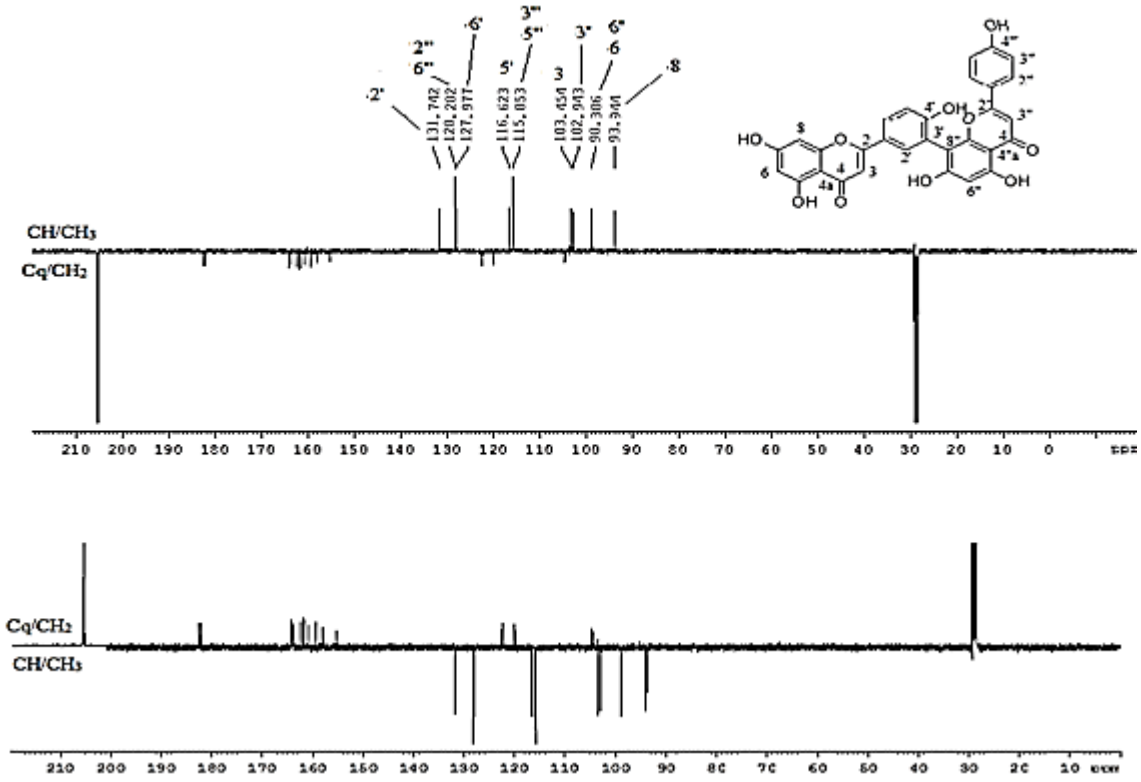
S32: IR spectrum of Amentoflavone **(7)**



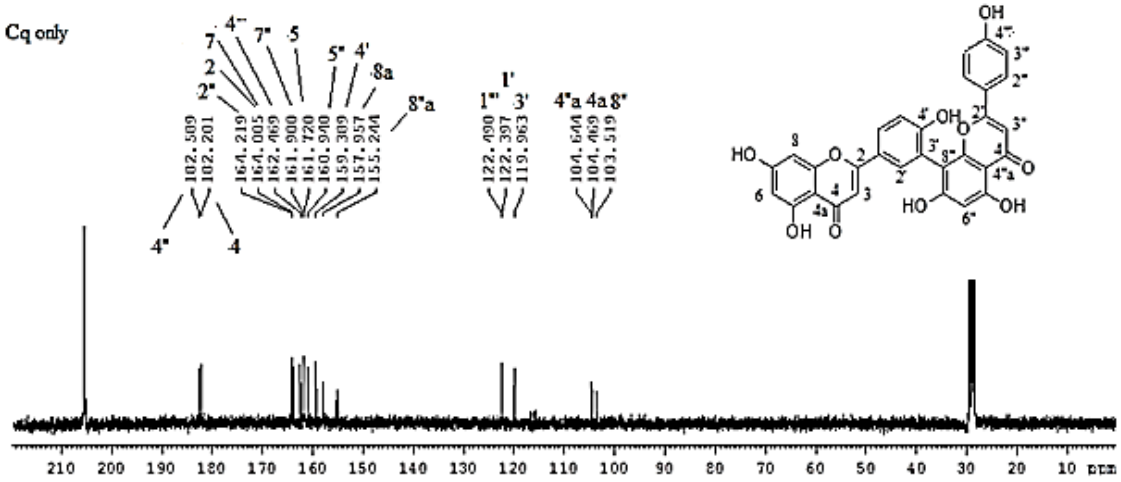
S33: ^{1}H NMR of Amentoflavone **(7)**



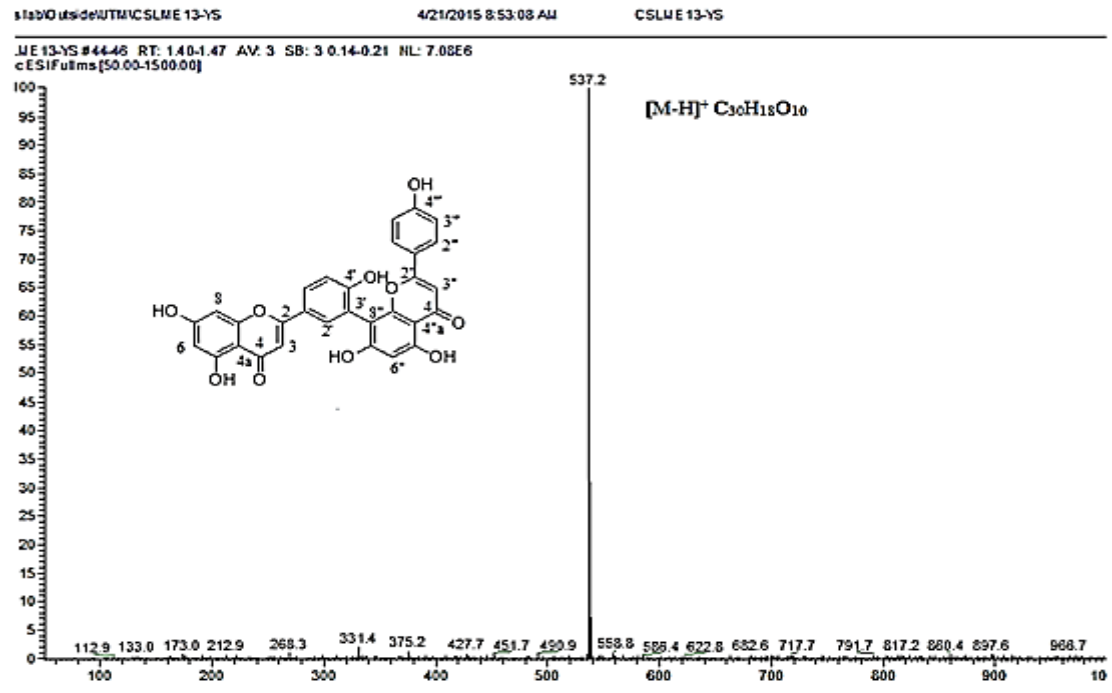
S34: ^{1}H NMR of Amentoflavone **(7)** (Expansion)



S35: DEPTQ spectra of Amentoflavone **(7)**



S36: DEPTQ-Q spectrum of Amentoflavone **(7)**



S37: ESIMS spectrum of Amentoflavone **(7)**

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